Analysis of the Effects of Short-Term Subaerial Weathering in Different Environmental Conditions On Bone Colour and Microstructure

by

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ABSTRACT:

This preliminary study was conducted in order to evaluate the effect of subaerial environmental factors on bone colour change over time, and to quantify this using photography and Photoshop© CMYK analysis, as well as with the Munsell Soil Colour Chart®. Additionally, surface microstructural changes with macroscopic and scanning electron microscopic (SEM) analysis were also conducted. Environmental data were tracked, and vapour density was documented and compared to relative humidity to determine the best-suited unit of measurement. The specimens consisted of 23 fleshed *Sus scrofa* humeri that were exposed to different microenvironments with varying substrates and sunlight exposure. Although no discernable colour change trends were observed, other taphonomic changes were visible macroscopically in as little as 6 weeks to a few months of environmental exposure, and detailed distinctions could be made between specimens of different groups using SEM analysis.

KEYWORDS: forensic science, forensic anthropology, forensic taphonomy, forensic ecology, dissection microscope, scanning electron microscope, vapour density, Photoshop©, Munsell Soil Colour Chart®

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Introduction

1.1 Introduction and Statement of Problem

Bone, being one of the strongest and most resilient biological materials in existence, comprises an important portion of biological forensic evidence, and is in some cases, the only form of evidence to persist. Hence, skeletal remains are sometimes used for postmortem interval (PMI) estimations, however, the conclusions drawn from these analyses are limited because there are so many variables involved in the decomposition of bone it is often difficult to determine the taphonomic history of bone based on its level of preservation. There has been a limited number of studies on developing an accurate and specific method for PMI estimation of skeletal remains, however these are usually done on non-fleshed specimens that have had years to decades of decomposition, and these studies are also looking exclusively at buried remains (1, 2, 3, 4). For these reasons, a short term analysis that would characterize how common environmental factors affect bone degradation of unburied fleshed bones would most likely be of interest to many forensic analysts, and the results found would present additional considerations for short PMI estimations.

1.2 Background Information

1.2.1 Bone Physiology and Composition

The anatomical microstructure of bone in key to our understanding of how certain factors contribute to the process of bone decomposition. Bone has a protein component (predominantly collagen) and a mineral component (hydroxyapatite)

that combine into a tissue that is both hard and elastic, making it resistant to degradation after death (5). Bone microstructure is determined by the organisation of the mineralised collagen fibrils, as well as bone vascularisation. In addition, the bone tissue is categorized in two principal forms: cancellous and compact bone tissues (6). Cancellous bone is composed of trabeculae, spicules of bone that support the red bone marrow, whereas compact bone is comprised of dense connective tissue that forms the cortex (6). Bone possesses a complex pore system of Haversian and Volkmann's canals, as well as other intricate anatomical components (Figure 1.1)(7). The central/Haversian canal is surrounded by concentric lamellae that together, make up an osteon. Volkmann's canals are also visible, and traverse the tissue perpendicular to the central canals. There are also interstitial lamellae, which are found between osteons, inner lamellae (nearest the medullary cavity) covered in a thin connective tissue called endosteum, and outer lamellae, which are likewise covered in a thin connective tissue called periosteum. Within the lamellae, there are small cavitations called lacunae, each containing an osteocyte (matured bone cell). Small canals, called canaliculi, transverse the bone tissue outwards from the lacunae (6).

In this current study, pig humeri will serve as test specimens. Domestic pig bones are effective analogues for human bones due to compositional similarities, and that they have a body mass greater than 5 kg (8).

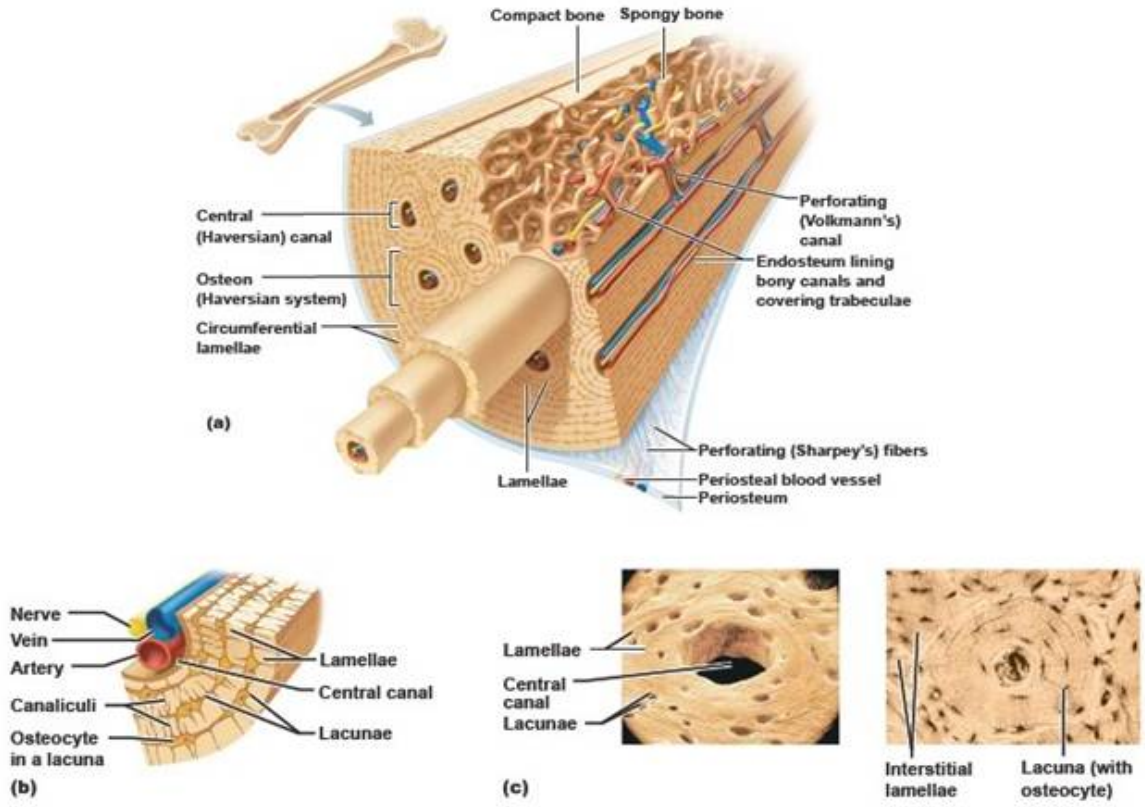

Figure 1.1: Bone Tissue Anatomy (7)

1.2.2. Speed and Cause of Postmortem Bone Changes

There is a vast array of fluctuating environmental factors that play a role in bone decomposition, and it is important to first understand the process of decomposition of bone before considering how environmental factors will have an effect on decomposition. To begin, the minerals in bone are held within the collagen matrix, and are protected from dissolution. Collagen is stable and insoluble in normal conditions, however, after death, slow chemical processes, such as hydrolysis, will begin to degrade the collagen matrix (9). This breakdown of the collagen matrix is part of a broader process known as diagenesis. There are only four general types of diagenetic pathways, despite the complexity of bone structure and the variability of the environment (Table 1.1). These pathways consist of the following: Type 1 - well preserved (WP), type 2 - accelerated collagen hydrolysis (ACH), type 3 - microbially attacked bone (MA), and type 4 - catastrophic mineral dissolution (10). Microbial alteration/bioerosion are common determinants of bone preservation (10).

Bioerosion is the result of bone microboring organisms degrading bone tissue. These organisms consist of fungi, bacteria, and in marine environments, cyanobacteria (9). Recent research has focused on how early these taphonomic factors lead to detectable bioerosion in an attempt to reveal predictable patterns and more accurate PMI estimations. Whether the bone specimen is fleshed or not has also been found to play a role in the rate of bone degradation. One

Table 1.1: Typical diagenetic parameter values for types of bone preservation described (5)

State of preservation	% 'collagen'	Infrared splitting factor	Carbonate/ phosphate ratio	Bulk density (g cm^{-3})	Skeletal density (g cm^{-3})	's' porosity	'm' porosity	'l' porosity	Histological Index	Cracking Index (%)
Modern bovine bone	22.7	2.8	0.43	1.9	2.1	0.0514	0.0235	0.0209	5	0
Well preserved	15+	3	0.4+	1.9	2.1	0.05–0.1	<0.1	0.02+	5	0
Catastrophic mineral dissolution	0–10	3.5–4.0	<0.2	1.2	1.2–3.0	0.1–0.4	0.1–0.3	0.06+	0–5	0–100
Accelerated collagen loss	<5	>3.9	0.1	1.0–1.5	2.5–3.0	0.3–0.4	<0.1	0.02+	5	100
Microbially attacked	0–20	3–4	0.3–0.4	1.5–1.8	2.3–2.5	0.05–0.25	0.2–0.3	0.02+	0–4	0–100

study focused on whether the principal forms of bioerosion found within the internal microstructure of human bone are produced by endogenous gut microbiota, or by exogenous bacteria from the soil (11). The early occurrence of bioerosion within the samples suggested that enteric putrefactive bacteria are primarily responsible for internal bone bioerosion (11). Diminishing the exposure of bone specimens to endogenous bacteria by having them removed from the carcass, as is done for the current study, will focus on environmental bioerosion rather than endogenous bioerosion.

Not only is environmental bioerosion a factor in bone degradation, the weather conditions in the general environment are as well, and they fluctuate constantly. It is for this reason that bone PMI estimations are often times very difficult to perform distinctly. The most consistently considered environmental factors include moisture, solar radiation, temperature, soil composition (containing a variety of microboring organisms and varying acidity and humidity levels), entomological factors (if there is any flesh remaining on the bone surface), and at times, scavenging.

Moisture has been observed to have varying effects on bone preservation; weathering in general appears to be slower in moist areas with vegetated cover, despite moisture being recognized as a factor involve in diagenesis of the bone due to hydrolysis of the collagen matrix of bone tissue (12). Moisture has been typically measured in the form of relative humidity, which is a percentage produced by dividing the amount of water in the air by the amount of water the air can hold at that given temperature (13). Because temperature is constantly fluctuating, it may

be crucial to consider other forms of moisture measurement that would better depict the amount of moisture in the air; vapour density, a measure of the grams of water in the air per cubic meter, may be a more appropriate candidate for representing air moisture measurements (13).

A bleaching effect on the surface colour of bone has been observed to begin immediately following exposure (12). Significant changes can be seen in as short of a time span as one month, and these changes may vary with humidity, since wet bone surfaces may reflect incident light (12). This colour change is typically quantified with the Munsell Soil Colour Charts®, and has also been previously quantified with digital spectroscopy (12).

Temperature has many effects not only directly on bone's structural integrity, but also on how other environmental factors may affect the bone. The direct role of temperature is through thermal expansion and contraction, which accumulates structural damage slowly over time, while sudden changes in temperature may induce fractures (12). This phenomenon may be intensified with the effects of freezing and thawing, especially if water is present within existing cracks. The expansion of ice will promote further cracking (12).

Additionally, temperature directly impacts the life cycle of entomologically relevant species, and at certain favourable temperatures, their activity is increased (14). The favourable temperatures would be expected to augment the rate of removal of flesh that may remain on the surface of the bone, thus exposing it to subaerial conditions. This will consequently affect the bone's exposure to other

decomposition factors of the environment, and ultimately increase the decomposition rate of the bone.

Lastly, soil composition has been found to have a significant impact on the type, rate, and extent of bone degradation (1). This is due to the variety of different soil types that vary by moisture levels, acidity, and content of microboring organisms.

1.2.3 Quantification of Bone Colour, Microstructure and Chemistry

Bone colour can be quantified in a few ways, one of which being the Munsell Soil Colour Charts. This system works by attributing a hue, value, and chroma measurement to the color of an area of the specimen by visual comparison to the predetermined colour plates in the chart (15). The hue is a measurement of colour from yellow to orange to red (particular to the soil chart), and is annotated with a numerical value that indicates the extent of red or yellow. For example, 10R is very red, 10Y is very yellow, 7YR indicates a colour that is between yellow and red, but is more yellow than red, whereas 2YR indicates a colour that is also between yellow and red, but is more red than yellow (15). The value is a measurement of brightness of the colour, and ranges numerically from 0-10 (from black to white). The chroma is the saturation of the colour, and is expressed on a scale that may vary in magnitude depending on colour. The attributed numerical value increases with colour intensity. The overall annotation is presented as “hue:value/chroma”, for example: 5YR:3/7. Another way that colour can be quantified is photographically; by photographing a specimen under controlled lighting conditions and uploading

the photo to Photoshop©, you can obtain a numerical colour measurement with the Colour Sampler tool (16).

To examine bone surface macrostructure as a means of characterizing degradation, a bone can be placed directly under a dissection microscope. One would be searching for indicators of weathering, such as cracking and exfoliation, which is when the cortex of the bone flakes off in layers (12). For increased magnification, a histological slide can be prepared for viewing under a light microscope. For an even greater depth of field and higher image quality, a specimen can be cut from the bone and prepared for scanning electron microscopy. The magnification obtained through the histological and SEM methods are required in order to determine which type of organism contributed to bone bioerosion, for at this level of magnification, it is possible to differentiate between fungal and bacterial alterations (9).

One method of quantifying diagenesis is through collagen extraction. The extraction process is accomplished by using a mildly acidic solution to solubilize the inorganic matrix; once eliminated, refluxing in distilled water will produced water-soluble collage.. The amount of collagen obtained from weathered bone may be compared to a standard of non-weathered specimens.

1.3 Goals of Study

The current abilities of forensic analysts to make distinct PMI estimations from skeletal remains is limited due to the difficulty of accurately taking into account every environmental factor that may have affected the degree of

decomposition of the remains. Additionally, there have been a limited number of studies conducted on bone decomposition, and these studies typically only considered non-fleshed buried specimens over extensive periods of time (1-4). This paucity in the literature necessitates a short-term (weeks instead of months or years) study conducted on fleshed bones on the surface, while tracking environmental data. The assessed condition of the bones and the different environmental factors would then be compared to note any trends. It may also help bring to light further considerations in making PMI estimations. Hence, this study attempts to examine the following:

1. Quantify the effect of sun exposure on bone colour change.
2. Compare bone surface microstructure from bones on different substrates.
3. Track environmental factors, such as temperature and moisture, and investigate possible correlations with decomposition.
4. Assess bone colour and surface microstructure alteration after exposure to a winter environment (buried in snow).
5. Investigate if either relative humidity or vapour density measurements have any bearing on decomposition.
6. Consider and characterize any observed bioerosion
7. Assess how these results may contribute to PMI estimations of skeletal remains.

Materials and Methods

2.1 Test Specimens

Twenty-four partially defleshed domestic pig (*Sus scrofa*) humeri were obtained from a local meat market in Sudbury, Ontario. These bones were stored in a freezer for approximately two weeks at most at the meat market, and subsequently in the Department of Forensic Science at Laurentian University until required. The humeri had minimal residual flesh, and an intact periosteum. The specimens were numbered one through 24 initially with a permanent marker on the humeral head, and later with a waterproof tag and fishing line tied through a drilled hole in the olecranon fossa of each respective specimen (Fig. 2.1). One area of the bone was selected for weathering and sunlight exposure analysis (Figs. 2.2-2.4).

2.2 Environmental Test Area

The bones were subject to natural weathering conditions in a designated decomposition area on the Laurentian University Campus (Fig. 2.5). This location provided a variety of differing microenvironmental conditions, such as fluctuating temperatures, vapour density, and areas of differing sunlight exposure, as well as different substrates of slightly varying topologies. These variables were monitored throughout the groups' exposure. The control group was kept indoors in the Forensic Osteology Laboratory on a paper substrate on a counter at a fairly constant and monitored temperature, vapour density, and with no exposure to direct sunlight.

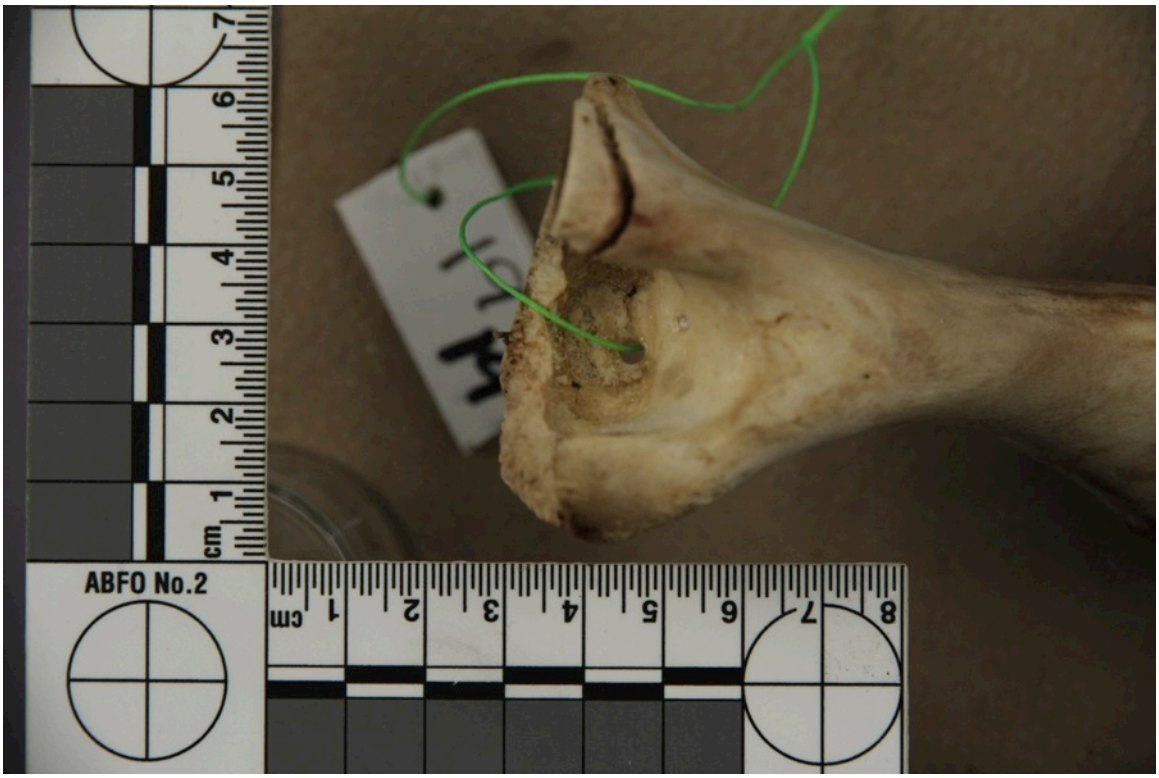

Fig. 2.1: Labeling of bone through olecranon fossa.

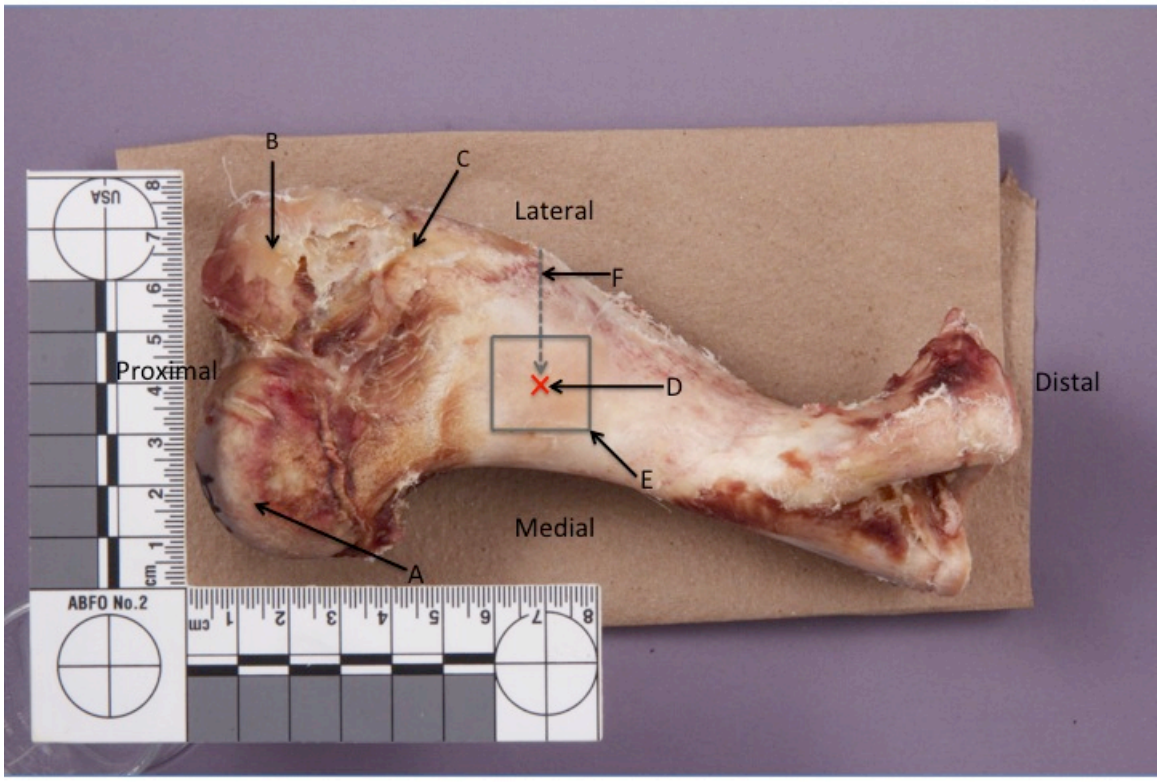

Figure 2.2: Diagram defining the area of analysis from the posterior view of a right humerus. A) Humeral head, B) major lateral tuberosity, C) deltoid tuberosity, D) central point of area to be examined, E) 2cm^2 area to be examined, F) 3.5cm distance beginning on lateral side (not visible), (Photo by C. Bouzane).

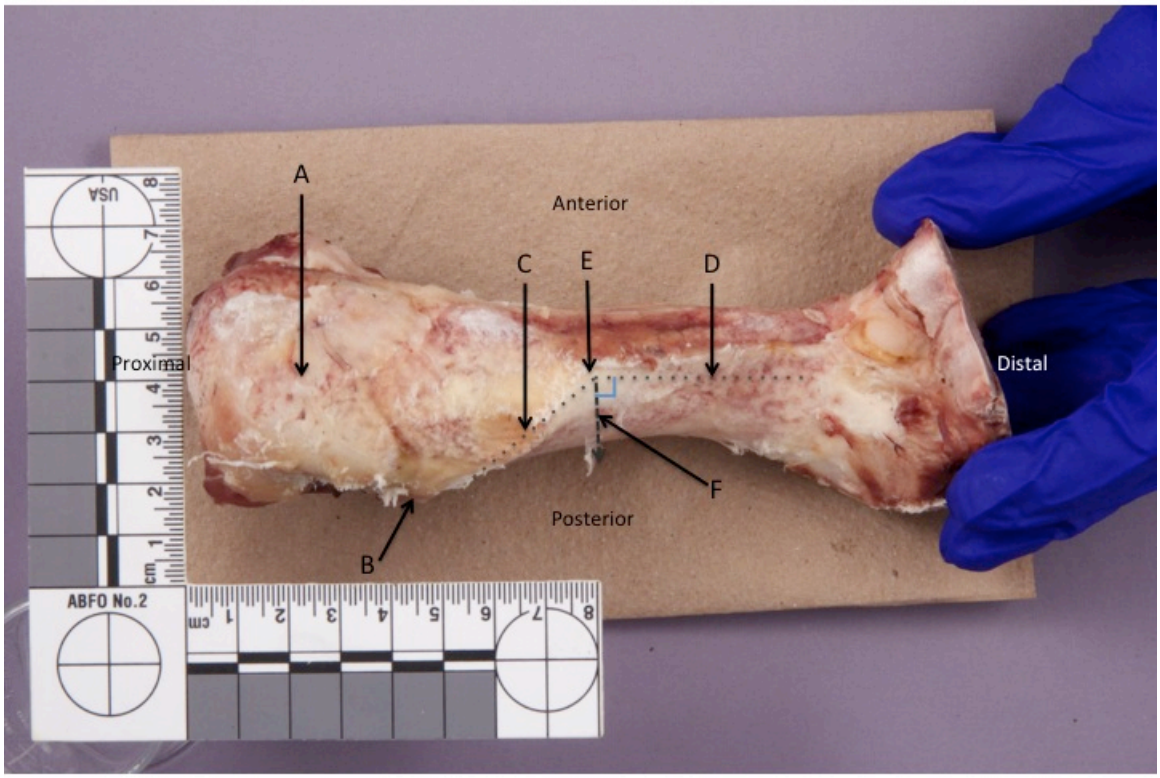

Figure 2.3: Diagram defining the area of analysis from the lateral view of a right humerus. A) Major lateral tuberosity, B) deltoid tuberosity, C) linear path of deltoid tuberosity, D) linear path of lateral diaphyseal crest, E) apex of angle between C and D, F) 3.5cm distance perpendicular to D beginning at E and continuing to the central point of area to be examined on posterior side (not visible), (Photo by C. Bouzane).

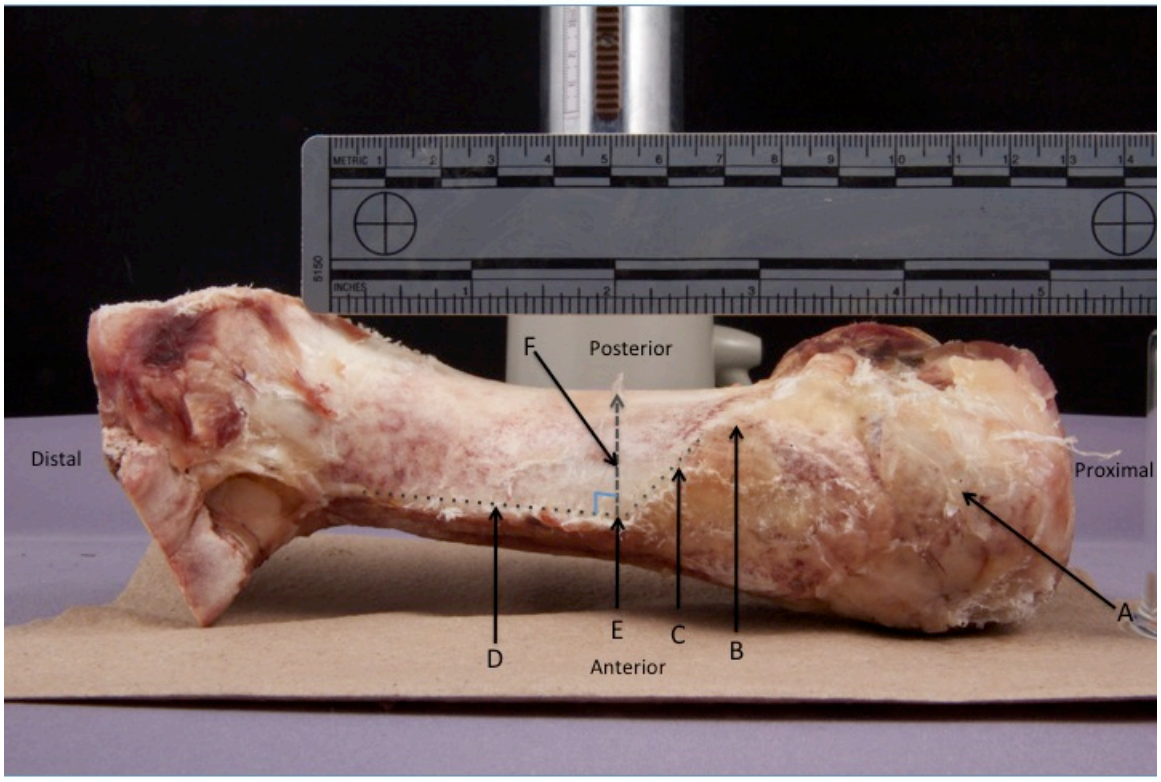

Figure 2.4: Diagram defining the area of analysis from an alternate lateral view of a right humerus. A) Major lateral tuberosity, B) deltoid tuberosity, C) linear path of deltoid tuberosity, D) linear path of lateral diaphyseal crest, E) apex of angle between C and D, F) 3.5cm distance perpendicular to D beginning at E and continuing to the central point of area to be examined on posterior side (not visible), (Photo by C. Bouzane).

Figure 2.5: The study site used on the Laurentian University Campus

2.3 Test Groups

There is a total of six groups numbered 1 through 6, consisting of four bones each. Groups 1 (Figure 2.6) and 2 (Figure 2.7) were analyzed to compare the effect of sun exposure on the colour change of bone over time, and to evaluate whether any bone bleaching was noticeable in the time frame of their environmental exposure. Group one, the sun exposed group, was placed in direct sunlight, whereas group two, was placed in the shade of a conifer tree. The latter was done in order to avoid sun exposure even after the falling of leaves. Both groups of bones were placed in cages pegged to the ground with a secured lid to curtail scavenging. To ensure group one was indeed exposed to more solar radiation than group two, a radiometer was used on both a sunny and shaded day at approximately 1pm (when the sun's rays are at their strongest) to measure the amount of solar radiation in watts per square meter being absorbed from each respective area, and to ensure group one was always exposed to more solar radiation than group two. Group three (Figure 2.8 and 2.9), group four (Figure 2.10 and 2.11), and group five (Figure 2.12 and 2.13) were all analyzed to compare the effects of varying substrate types on bone surface microstructural integrity. As a result, group three bones were placed on a rock substrate, group four bones were placed on a moist soil substrate, and group five bones were placed on a grass substrate. Initially, to increase contact between the substrate and the bone, the cages were placed upside down without a

Figure 2.6: Set-up of the sun exposed group 1 bones.

Figure 2.7: Set-up of the shaded group 2 bones.

Figure 2.8: Set-up of the rock substrate group 3.

Figure 2.9: Close-up of cage 1 containing specimen 11 (top) facing west, and 12 (bottom) facing east.

Figure 2.10: Set-up of the moist soil substrate group 4.

Figure 2.11: Close-up of cage 7 containing specimen 15 (top) facing west, and 16 (bottom) facing east.

Figure 2.12: Set-up of the grass substrate group 5.

Figure 2.13: Close-up of cage 5 containing specimen 19 (top) facing west, and 20 (bottom) facing east.

lid and tied to pegs nailed in the ground. After a week or so, specimen fourteen from group four (moist soil group) was scavenged, and other similarly placed cages had displaced bones (Figure 2.14). To avoid future scavenging, the cages were pegged right side up to the ground with secured lids and a portion of the corresponding substrate was placed in the bottom of the cage, as is depicted in the previous Figures. The missing specimen fourteen was replaced with control group specimen twenty-one. The control group, as previously mentioned, remained in the laboratory on a paper substrate out of any sunlight and at a reasonably consistent monitored temperature averaging 23°C (Figure 2.15). A summary table of the groups with their specimens and cage ID numbers can be found in Table 2.1. Once the weather no longer permitted the continuation of exposure to the desired environmental factors due to snowfall, the bones of groups three, four and five were brought inside in labeled bags and stored in the refrigerator in the Department of Forensic Science for a period of roughly 4 weeks, whereas groups one and two were left outside in the rain and in the snow for a period of roughly 11 weeks to analyze the effects of a winter environment on bone surface structure integrity. Group one (sun exposed) and two (shaded) were to be left outdoors in the snow for winter environment exposure, principally because they had been periodically photographed, and those photos taken are sufficient for bone colour change analysis (see below). The winter environment setup (Figure 2.16) consisted of a snow stick to measure snow height, and an apparatus that held two thermocouples in place on the surface and bone-ground-interface of one bone

Figure 2.14: Photo of some of the scavenging from the moist soil group 4.

Figure 2.15: Control bones placed on paper on a counter in the Osteology Laboratory.

Table 2.1 Summary of Groups, Specimens, Cages, and Bone Orientation

Group	Cage ID #	Specimen #	Bone Orientation
1	6	1	West
1	6	2	East
1	2	3	West
1	2	4	East
2	9	5	West
2	9	6	East
2	3	7	West
2	3	8	East
3	4	9	West
3	4	10	East
3	1	11	West
3	1	12	East
4	8	13	West
4	8	14 <-> 21*	East
4	7	15	West
4	7	16	East
5	16	17	West
5	16	18	East
5	18	19	West
5	18	20	East
6	N/A	22	N/A
6	N/A	23	N/A
6	N/A	24	N/A

*After scavenging, missing specimen 14 was exchanged for control bone 21.

Figure 2.16: Example of winter environment setup for group 1. The same was done for group 2.

from each group. To protect the thermocouples from water damage, a plastic bag was secured around them, and the thermocouples were wrapped around a pole that was secured in the ground.

2.4 Tracking Specimens

Although each specimen was initially labelled from one to twenty-four with a permanent marker (sharpie TM) on the humeral head, the ink eventually faded. Upon initial placement in the cages, the bones were positioned posterior side up, with their proximal ends either facing east or west in the cages, which were themselves oriented north. Only two bones (facing either east or west) were placed in each cage, and there were two cages to accommodate the four bones per group. The orientation of specimens, along with their cage and group number, was documented before hand (Table 2.1). Once snowfall commenced on November 21st 2015, all specimens were brought indoors in plastic bags, with the exception of those left for winter exposure (group one and two). Later on, the specimens from groups three, four, and five were gently rinsed under warm water to remove mould or loosened flesh that was falling off, then further analyzed for microstructural changes with a dissection microscope. They were then tagged by drilling a hole into the olecranon fossae of each bone, and a plastic label with the specimen number, written in both permanent marker and wax pencil, was affixed using fishing line (Figure 2.1). This new labelling facilitated tracking the specimens during chemical treatment, a process that will be further discussed later in this section.

2.5 Environmental Data

2.5.1 Temperature Measurement

Daily temperature measurements were taken consecutively for 43 days. Maximum and minimum temperatures were recorded with two max/min thermometers positioned each at the higher and lower topological region of the vicinity in order to obtain an accurate daily maximum and minimum temperature range. For each group, the following temperature measurements were recorded daily: ambient one foot, bone surface, ground, and bone-ground interface. The ambient temperature was also recorded at the time of data collection of the general environment at a location roughly equal distance from all five outdoor groups. The above-mentioned temperatures were recorded with digital Omega thermometers (version HH-25TC, number T-294631, Omega Engineering Inc., 1 Omega Dr, Stamford, CT 06907, United States) attached to a thermocouple. The end of this thermocouple was taped to a meter stick so that body heat would not affect the temperature reading, and a shadow was cast upon the tip of the thermocouple to ensure the sun's radiation did not conduct any heat onto it. Similar temperature measurements were taken of the control group in the laboratory.

2.5.2 Winter Temperature Measurements

Bone surface and bone-ground interface temperatures were recorded weekly with the Omega digital thermometer from the thermocouples that were set upon metal poles hammered into the ground (see Figure 2.16). Snow height levels were also recorded in centimetres with a snow stick that was hammered into the ground

prior to snowfall. This was only done for groups one and two that were left outdoors after snowfall.

2.5.3 Relative Humidity and Vapour Density Measurement

Initially, the relative humidity was measured daily using an Assman Aspirating Cycrometer (number 4510, Casella London Ltd., Lee Valley Technopark, Ashley Rd, N17 9LN, London, UK). This apparatus records wet and dry bulb temperatures in degrees Fahrenheit, and these measurements are converted to relative humidity measurements. A few weeks into data collection, the Aspirating Cycrometer was replaced with a newly obtained kestrel 3000 weather meter (number 1641052, Nielsen-Kellerman, 21 Creek Circle, Boothwyn, PA 19061, US) that directly records relative humidity with no need of conversion. The aforementioned relative humidity measurements were taken at a location roughly equidistant from all five outdoor groups, and at each of the five outdoor groups as close to the bone surface as possible. This procedure was repeated for the control group in the laboratory. Vapour density values, in grams per cubic meter, were derived from the relative humidity and temperature values using a Vapour Density Chart (13) (Figure 2.17).

2.6 Analysis of Effect of Sun Exposure

2.6.1 Schedule and Organization of Photographic Analysis

Specimens from groups one (sun exposed) and two (shaded) were brought into the lab twice a week for photography. The control group specimens

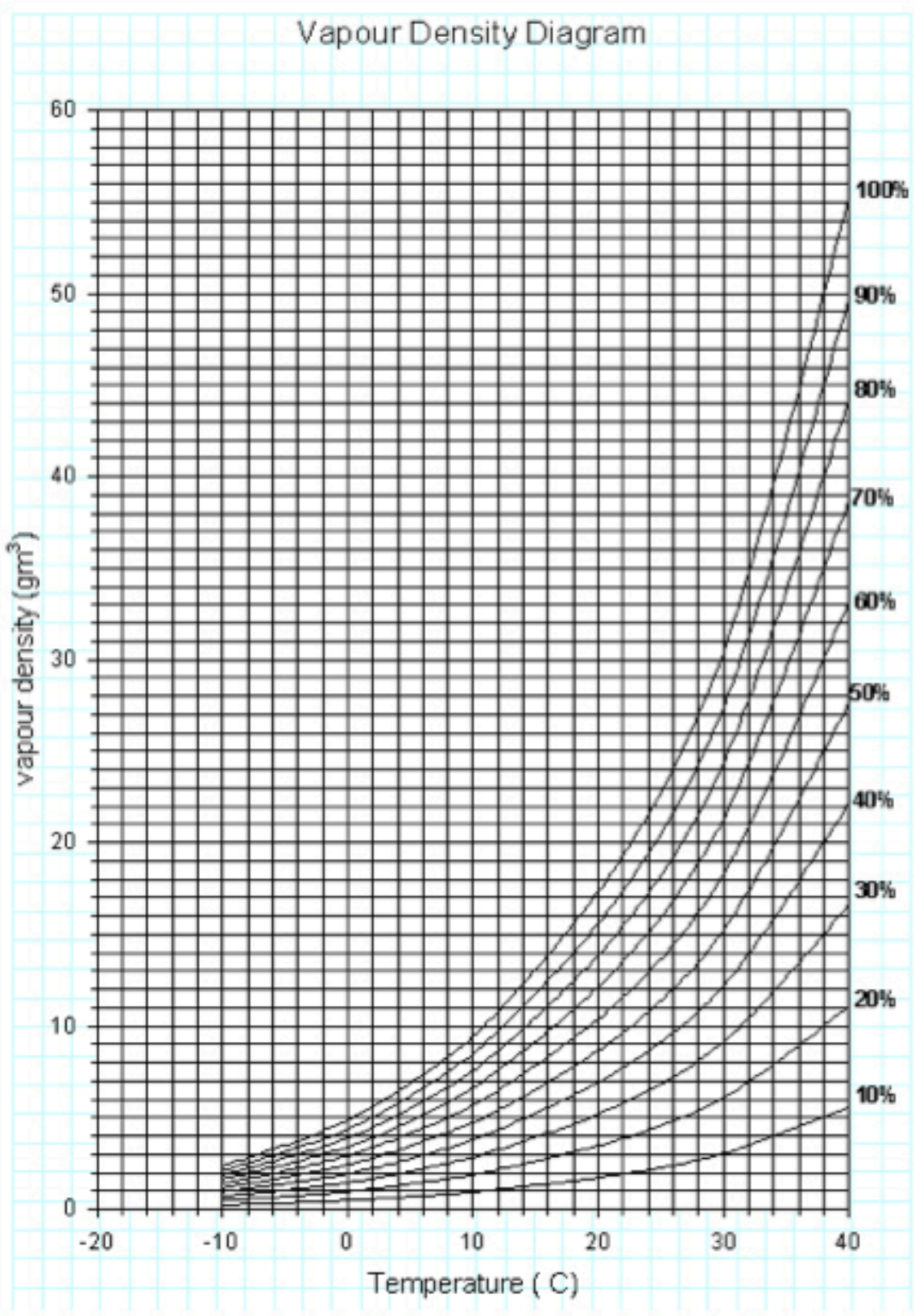

Figure 2.17: Chart used for conversion of relative humidity to vapour density(13).

were also photographed on the same schedule for comparison. Outdoor groups one and two were transported in labeled and sealed plastic bags in order to reduce their exposure to the indoor environment. A digital SLR camera (Canon© 60D©, Canon Inc., Mississauga, Ontario) was used to photograph every specimen with a wide-angle lens under standardized lighting conditions using two lamps kept at the same angle to reduce shadows in combination with even light dispersal using a white curtain, as well as with an ABFO No. 2 scale (Figure 2.18). In addition to the generic photographs (Figure 2.19) that were taken with the wide-angle lens, a macro lens was also used to obtain detailed photographs of every specimen at the proximal (Figure 2.20) and distal ends (Fig. 2.21). Each photograph captured the specimens' posterior aspect. The camera was positioned with a level perpendicular to the specimen. Every photo taken over the given period of time was done under standardized conditions; the same two lamps were positioned at the same angle and the resulting incident light was diffused using a photography box with a white curtain to reduce shadows and even the lighting. The camera was set to ISO 400, with an aperture ƒ/25. The camera was white balanced using the same standard card each time.

2.6.2 Quantifying Colour Change

The colour change of bone over time recorded photographically of groups one, two and the control group was quantified by using Adobe Photoshop© (version CS2, Adobe Systems Inc., San Jose California) to obtain CMYK values of each photo of

Figure 2.18: Photography setup in the Osteology lab.

Figure 2.19: Example of one of the standard photos of specimen 1 taken with the wide angled lens on November 19th, 2015.

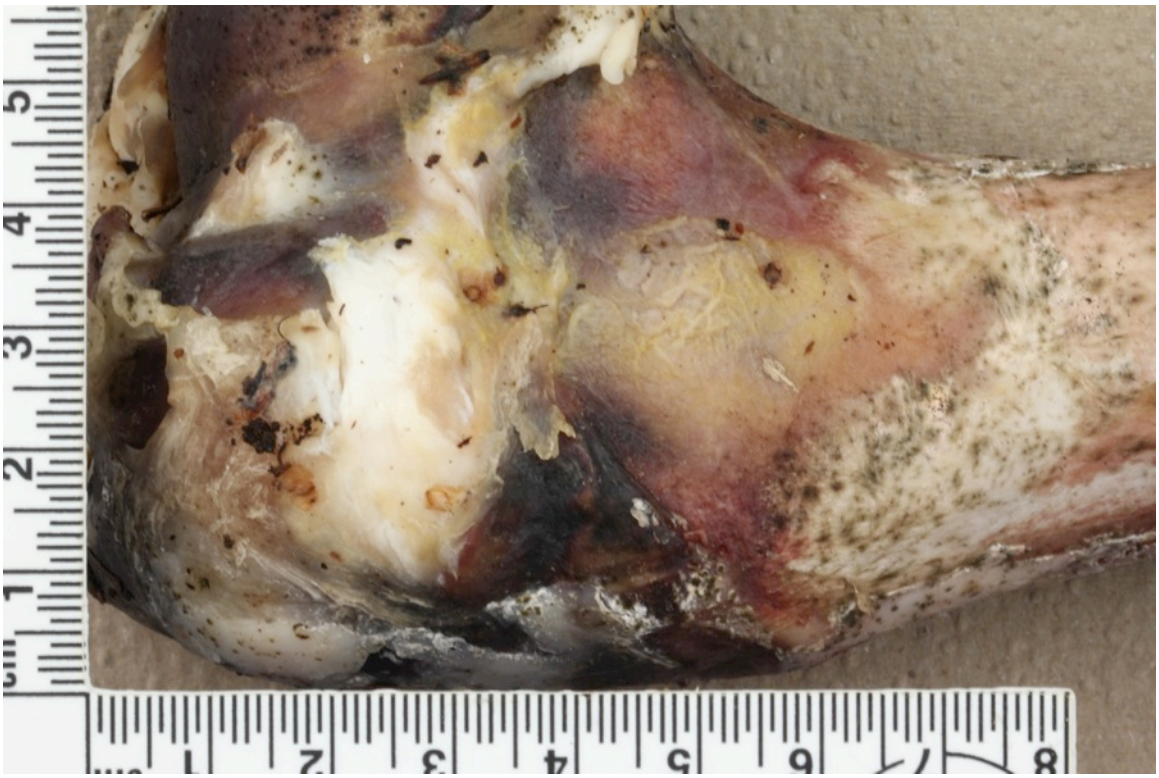

Figure 2.20: The proximal end of specimen 1 photographed with a macro lens taken on November 19th, 2015.

Figure 2.21: The distal end specimen 1 photographed using a macro lens on November 19th, 2015.

every specimen. Since there was a range of colours in the area to be analyzed on the diaphysis of the bone, a range of CMYK values was obtained for five points within the defined area of analysis that incorporated the brightest and darkest points. In addition to this, every specimen's area of analysis colour was quantified with the Munsell Soil Colour Chart (15), and subsequently photographed beside the page of the Munsell Soil Colour Chart that contained it's corresponding colours. Regression analysis was used to illustrate the observed colour change (CMYK values) over time. Seriating the bones from lightest to darkest was also performed and photographed.

2.7 Analysis of Substrate Effect

2.7.1 Removal of Soft Tissue

Initially, the specimens were rinsed under warm water and gently rubbed with a toothbrush to remove pieces of rotted flesh that were falling off, as well as mould. Care was taken in order to ensure the periosteum, if remaining, remained intact after this initial rinse. This was also later done to the winter environment bones from group 1 and 2 that were left outdoors, and the control group 6 bones. All of the specimens were subsequently analyzed macroscopically, assigned a Munsell Soil Chart colour, and photographed (Figure 2.22). Afterwards, they all underwent chemical maceration in a Tergazyme solution (100mL to 20L of water), for four to eight hours in the solution at temperatures ranging from 65°-90°C. This was monitored with a glass thermometer. It is suggested to maintain the solution at a temperature of 60-80°C. Once the soft tissue had been removed from each

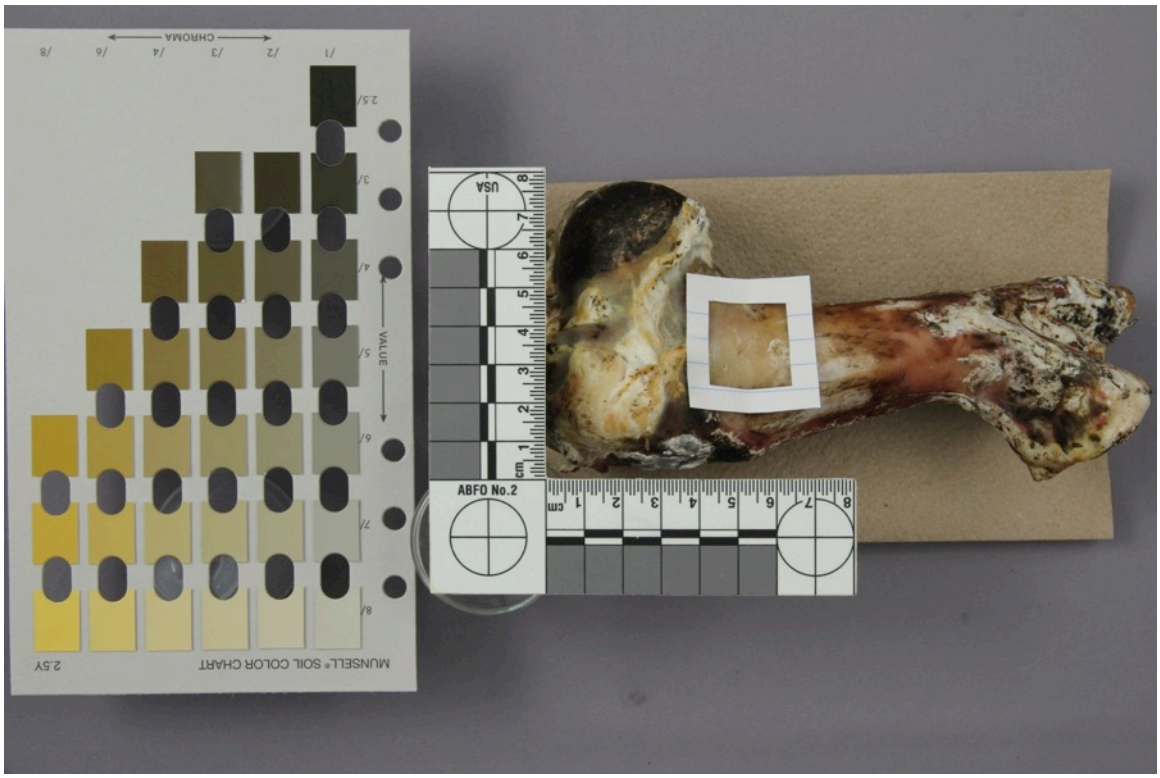

Figure 2.22: Standard photo of specimen 1 with corresponding Munsell Soil Colour Chart.

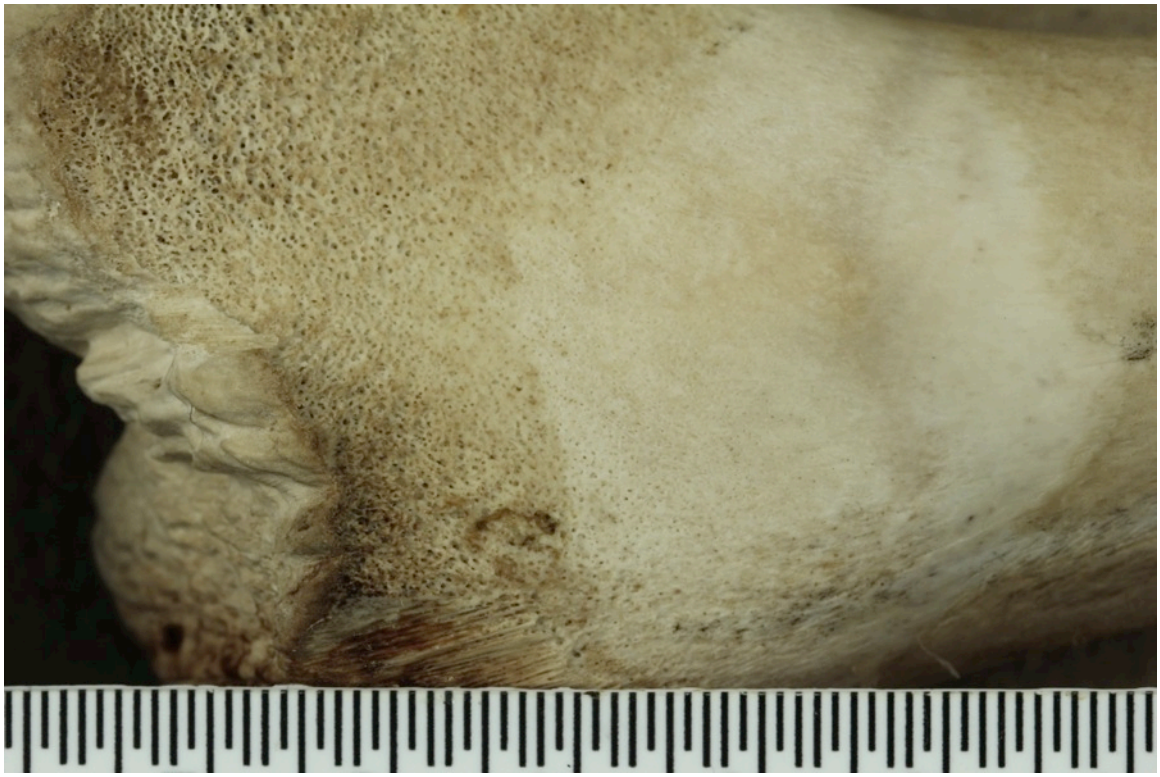

Figure 2.23: Macroscopic photo taken after maceration of the area of analysis of specimen 1.

specimen, they were subsequently re-examined and photographed as above (Figure 2.23).

2.7.2 Dissection Scope Analysis

A dissection microscope (number SZ-STB1, Olympus, Japan) was used to examine every specimen in the area demonstrated in Figure 2.2. The key features that were noted included: presence/absence of bone cracking along with the number of cracks (if present), presence/absence of bone flaking along with whether flaking (if present) covered >/<50% of the area of analysis, presence/absence of periosteum along with whether periosteum (if present) covered >/<50% of the area of analysis, and any other notable features. A Munsell Soil Chart colour was also assigned to each area of analysis, and in some cases more than one colour was assigned.

2.7.3 Scanning Electron Microscopy

Only certain representative specimens or those that exhibited interesting weathering, along with a control specimen, were chosen for SEM analysis for microscopic comparison of surface microstructures. Those chosen are outlined in table 2.2. The area of analysis previously defined was cut out of the bone's diaphysis with a hand-held rotary saw under a fume hood. Although the specimens had previously been macerated in the heated Tergazyme solution, upon cutting into the medullary cavities of some specimens, marrow still remained and coated the inner surfaces of some excised specimens. These specimens were subsequently treated with the Tergazyme solution. All excised specimens were then rinsed once more and gently scrubbed with a toothbrush under warm water to remove any grease from

the inner surface, and others if needed had the trabecular surface filed down flat with the rotary saw to ease sample mounting. After rinsing, specimens were dried in an oven at 80°C to mounting for SEM analysis.

The mounting of the specimens was accomplished by securing each to a 12mm diameter carbon adhesive disk that was secured to a corresponding aluminum stud with the same diameter. Colloidal silver paint was additionally added for adhesive purposes and also to aid in completing the circuit from the specimen's superior surface, to the carbon disk, to the aluminum stud. Once the paint had dried, specimens were coated with approximately 10nm of gold using a Cressington Sputter Coater (number 108, Cressington Scientific Instruments Ltd., 34 Chalk Hill, Watford WD19 4BX, England, UK) to produce the required conductive surface for the SEM.

All prepared specimens were then visualized using a Cambridge Stereoscan scanning electron microscope (number 120, Cambridge Scientific, 199 Dexter Avenue, Watertown, MA 02472). Several micrographs were captured as jpeg files to record the total surface area of each specimen. Subsequent photos were taken of any notable features likely to be indicative of weathering including microfractures, exfoliation, or any evidence of potential bioerosion (using higher magnifications).

Table 2.2 Summary of specimens chosen for SEM analysis

Specimen Chosen	Reason
23	Significant flaking, and representative of control group
24	Was the palest of all specimens, and representative of control group
12	Representative of rock substrate group (3), experienced mould growth
16	Representative of moist soil substrate group (4), Likely experienced mould and bacterial growth
17	Representative of grass substrate group (5), also darkest specimen of all
18	Representative of grass substrate group (5), pronounced flaking and suspected mould growth
4	Representative of winter environment group (1), mould growth
7	Representative of winter environment group (2), mould growth

Results

3.1 Environmental Data

3.1.1 Relative Humidity and Vapour Density Data

During the environmental data collection, both vapour density and relative humidity were recorded throughout the environmental exposure of groups 1-6 for 43 days. These data were used not only to monitor the exposure conditions, but also to analyze the appropriateness of each as an indicator of humidity. A regression of daily relative humidity vs. daily temperature was compared to that of a regression of daily vapour density vs. daily temperature. A coefficient of determination of only 0.00054 was obtained for the relative humidity; however, a coefficient of determination of 0.71 was obtained for the vapour density (Figure 3.1 and Figure 3.2). This indicates that vapour density is already more easily predicted from temperature than relative humidity.

Additionally, the fluctuation of relative humidity and vapour density were graphed over the 43 days of data collection for both outdoor and indoor conditions. The relative humidity graph (Figure 3.3) illustrates in almost every instance higher relative humidity values outdoors than inside, however the vapour density graph (Figure 3.4) illustrates the opposite with almost always higher vapour density values inside than outdoors.

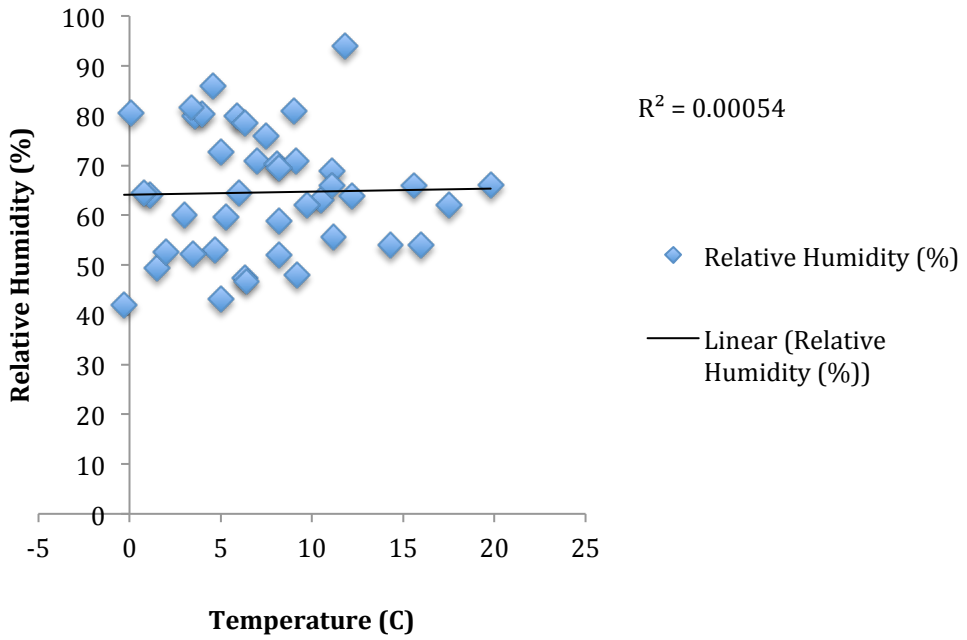

Figure 3.1: Regression of Relative Humidity (%) Vs. Temperature (°C)

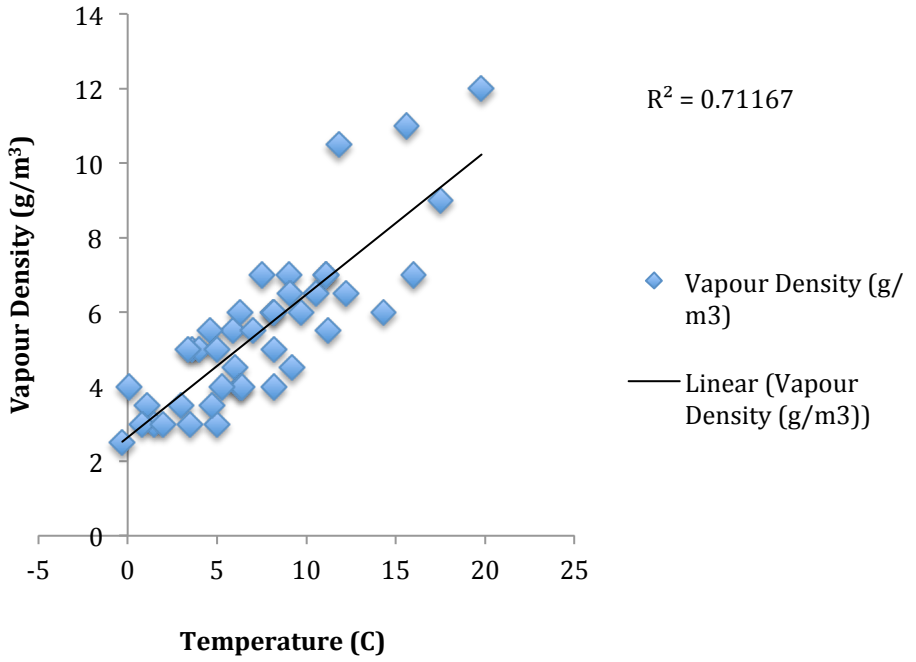

Figure 3.2: Regression of Vapour Density (%) Vs. Temperature (°C)

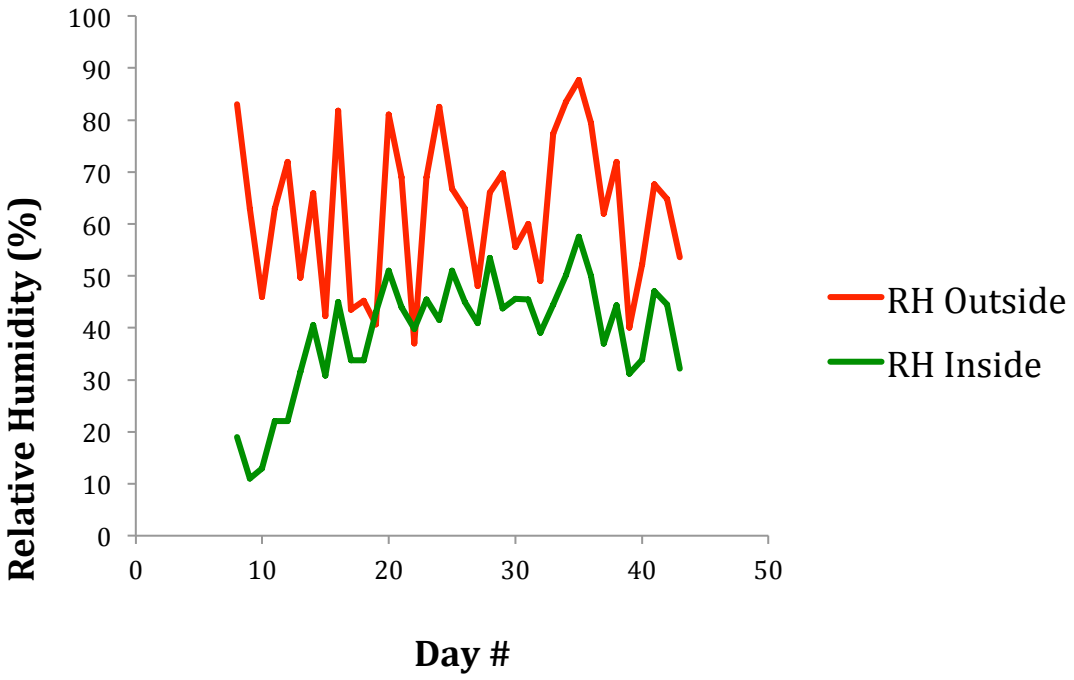

Figure 3.3: Outside and Inside Relative Humidity Values Over the Total 43 Days

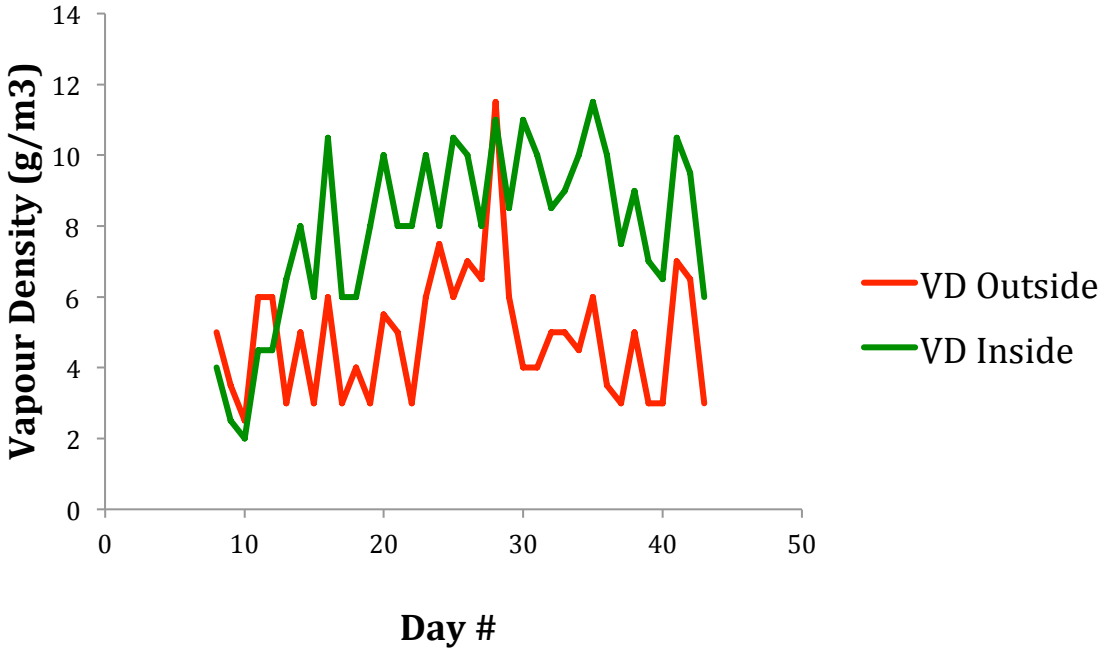

Figure 3.4: Outside and Inside Vapour Density Values Over the Total 43 Days

3.1.2 Weather Profiles for All Six Groups

Daily temperatures were collected to monitor temperature fluctuation during the environmental exposure of all 6 groups. These temperature profiles are graphed and represented in Figures 3.5 to 3.10.

3.1.3 Winter Environment Weather Profiles for Groups One and Two

After the initial 43 days (up until first snow fall), groups three, four, and five were brought indoors to prevent exposure to snow, whereas, groups one and two (initially used to track colour change from sun exposure) remained outdoors in the winter environment for eleven weeks, totalling their complete outdoor environmental exposure to 120 days. During these eleven winter weeks, there was significant temperature fluctuation and precipitation. Weather data obtained from The Weather Network (ultimately Environment Canada) during those eleven weeks is summarized in Table 3.1.

Table 3.1: Sudbury Weather Data During Winter Environment Exposure of Groups One and Two: November 21st, 2015 - February 6th, 2016

Maximum Temperature	13.3 °C
Maximum Temperature Date	December 24th, 2015
Minimum Temperature	-26.9 °C
Minimum Temperature Date	January 13th, 2016
Precipitation Accumulation	293.2 mm

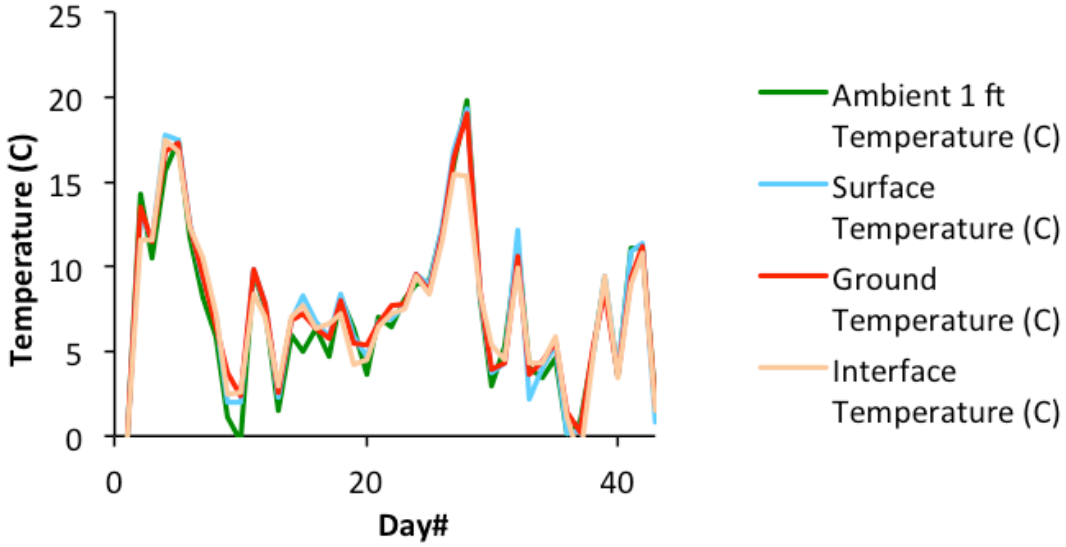

Figure 3.5: Temperature Profile of group one (sun exposed) during 43 days of environmental exposure

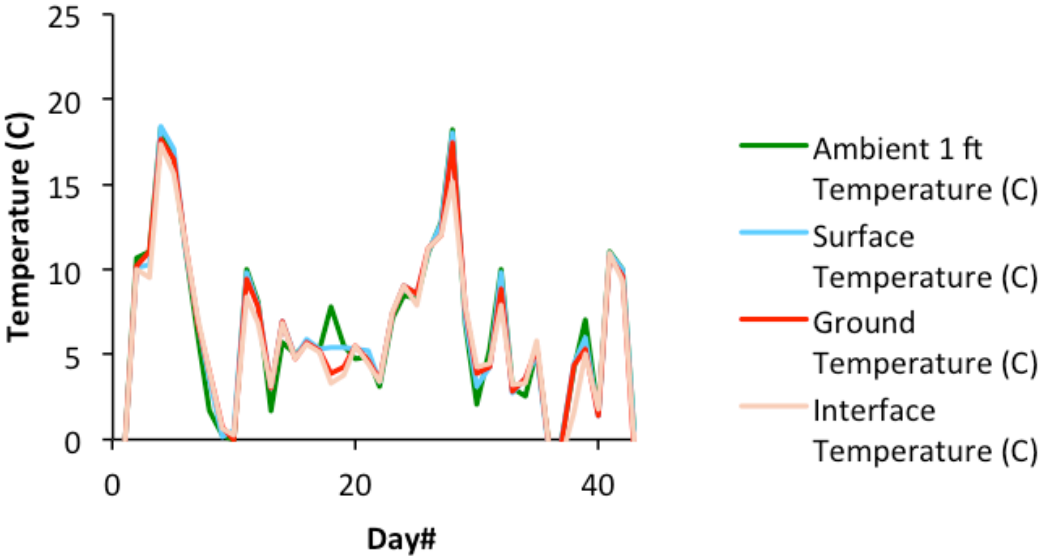

Figure 3.6: Temperature Profile of group two (shaded) during 43 days of environmental exposure

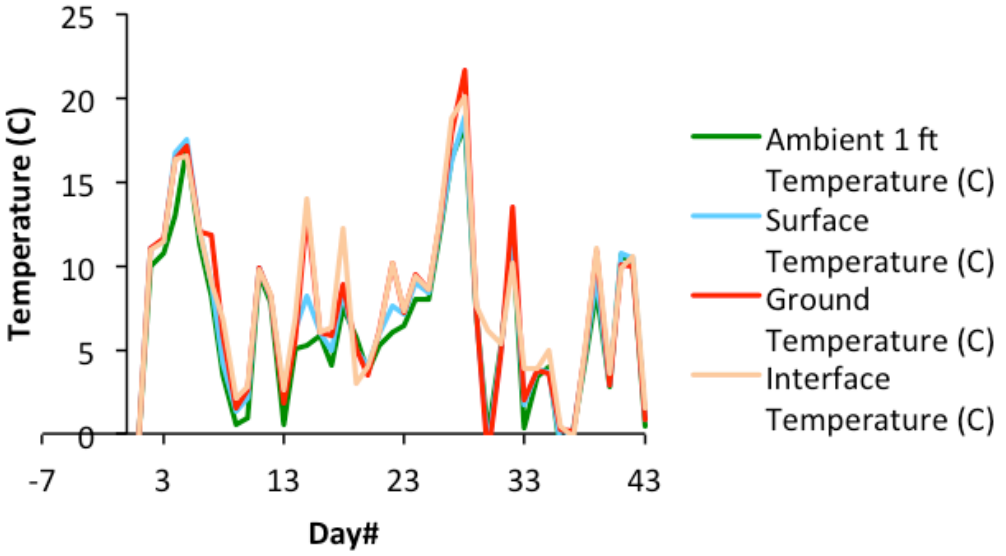

Figure 3.7: Temperature Profile of group three (rock substrate) during 43 days of environmental exposure

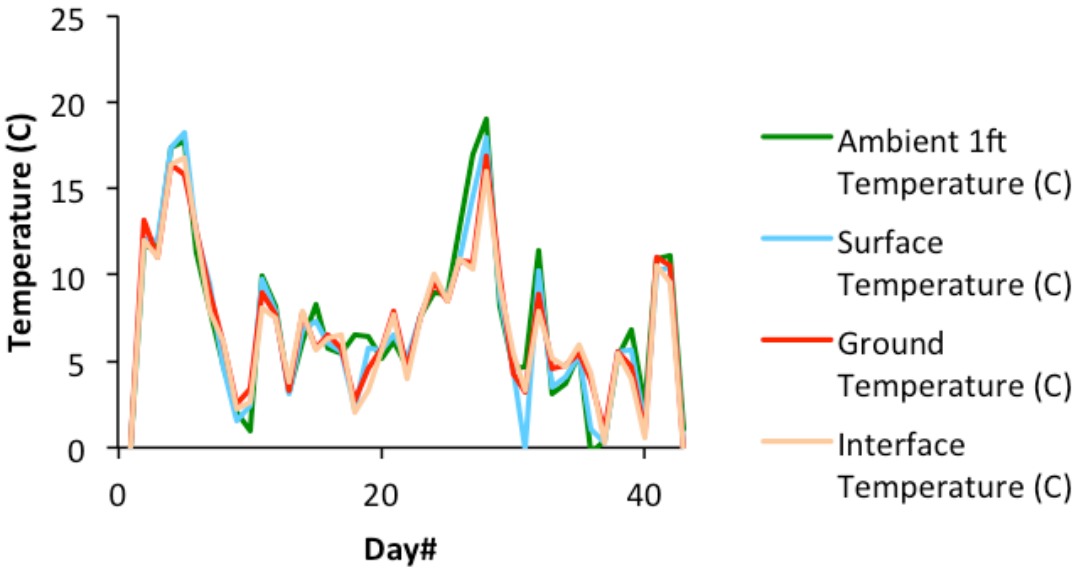

Figure 3.8: Temperature Profile of group four (moist soil substrate) during 43 days of environmental exposure

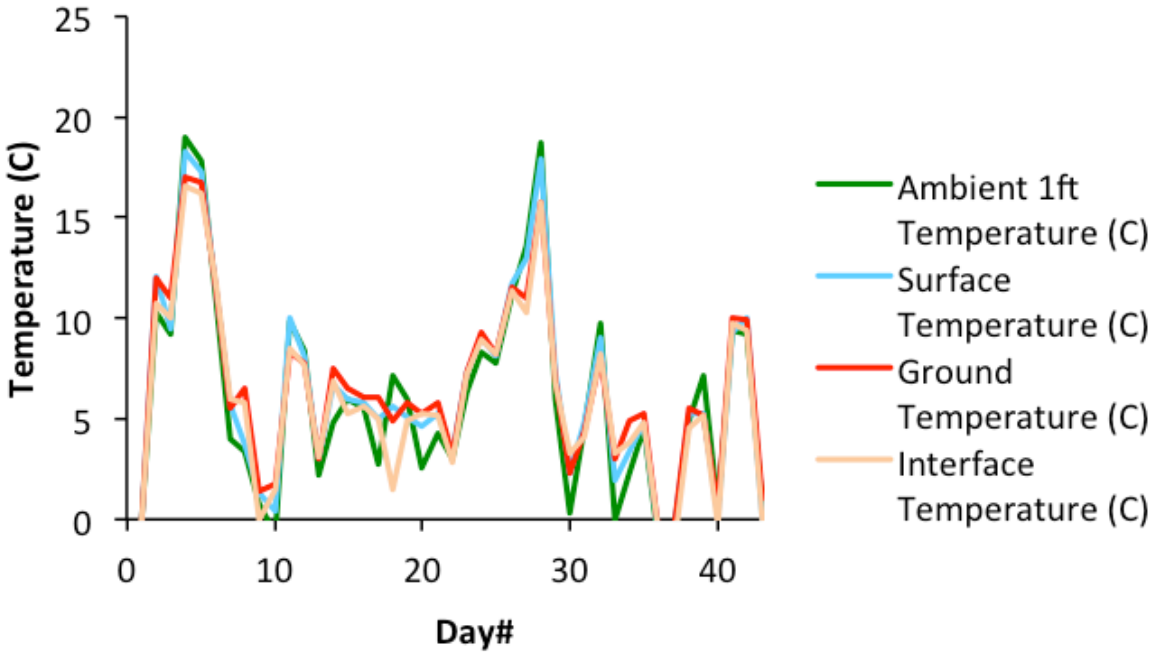

Figure 3.9: Temperature Profile of group five (grass substrate) during 43 days of environmental exposure

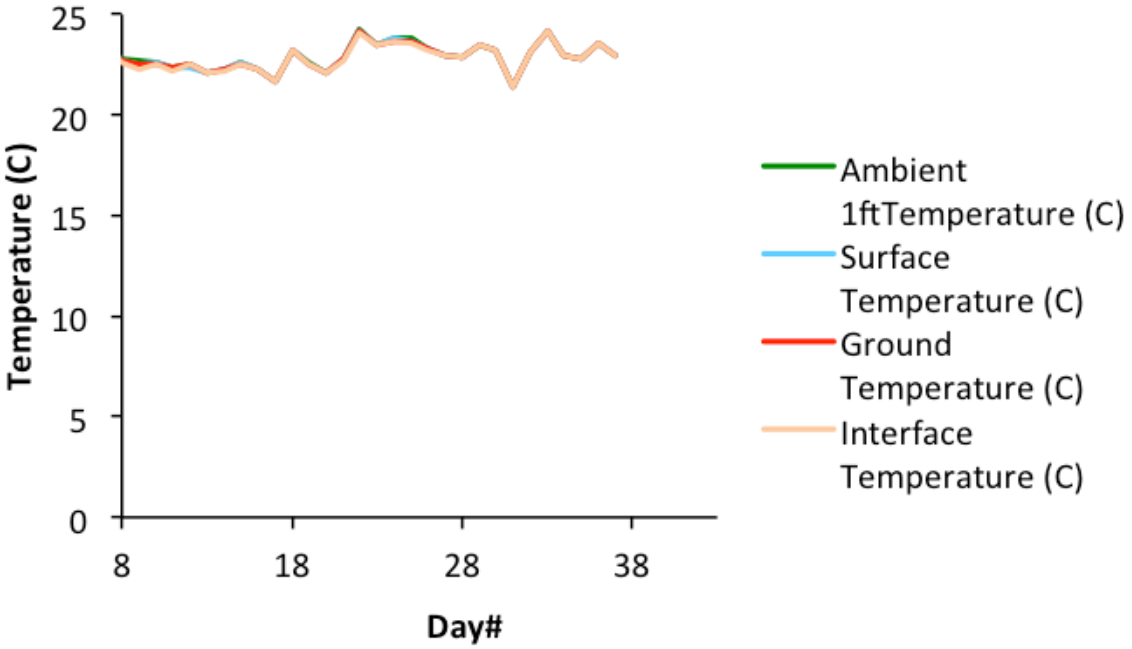

Figure 3.10: Temperature Profile of group six (control) during 43 days in the laboratory

3.2 Colour Change Due to Weathering

3.2.1 Regression Analysis of CMYK Data From Photos Taken Over Time

The first method of colour change quantification involved performing a linear regression for the cyan (Figure 3.11), black (Figure 3.12), yellow (Figure 3.13), and magenta (Figure 3.14) values obtained from the photos of the control group, the sun group, and the shaded group over time using Photoshop©. No strong linearity was observed as only low coefficients of determinations were obtained. The colour that appeared to have the strongest linear relationship over time, no matter the group, was the cyan with R^2 values of 0.52273 for the shaded group, 0.4315 for the control group, and 0.46179 for the sun group. Yellow, magenta and black each yielded less strong linear relationships. The next colour that generally showed the strongest linear relationship was the black colour with R^2 values of 0.09915 for the control group, 0.26159 for the shade group, and 0.29367 for the sun group. The cyan and black colour linear relationships had a positive slope suggesting that the bones, no matter what the group, took on slightly more cyan/black colours over time. Based only on these two colours as indicators of darkness, the darkest groups were always the outdoor groups, and the lightest the control group. The other two colours, magenta and yellow, showed very weak linear relationships that were either neutral (for yellow) or slightly negative (for magenta).

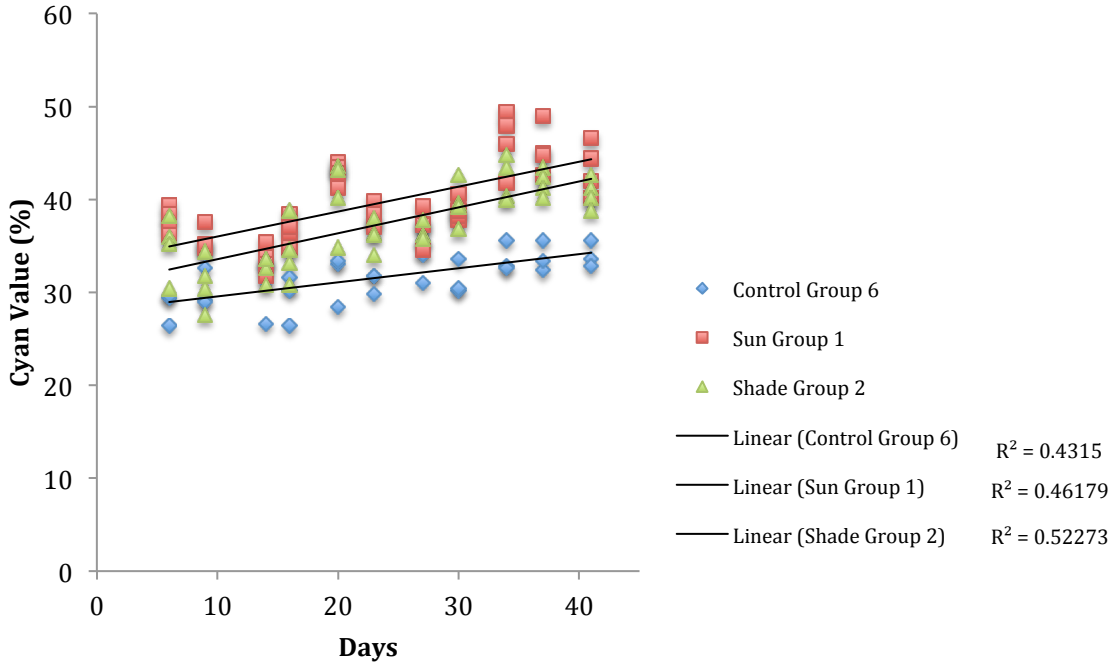

Figure 3.11: Cyan Values from Photos of Control Group 6, Sun Group 1, and Shade group 2 Over Time

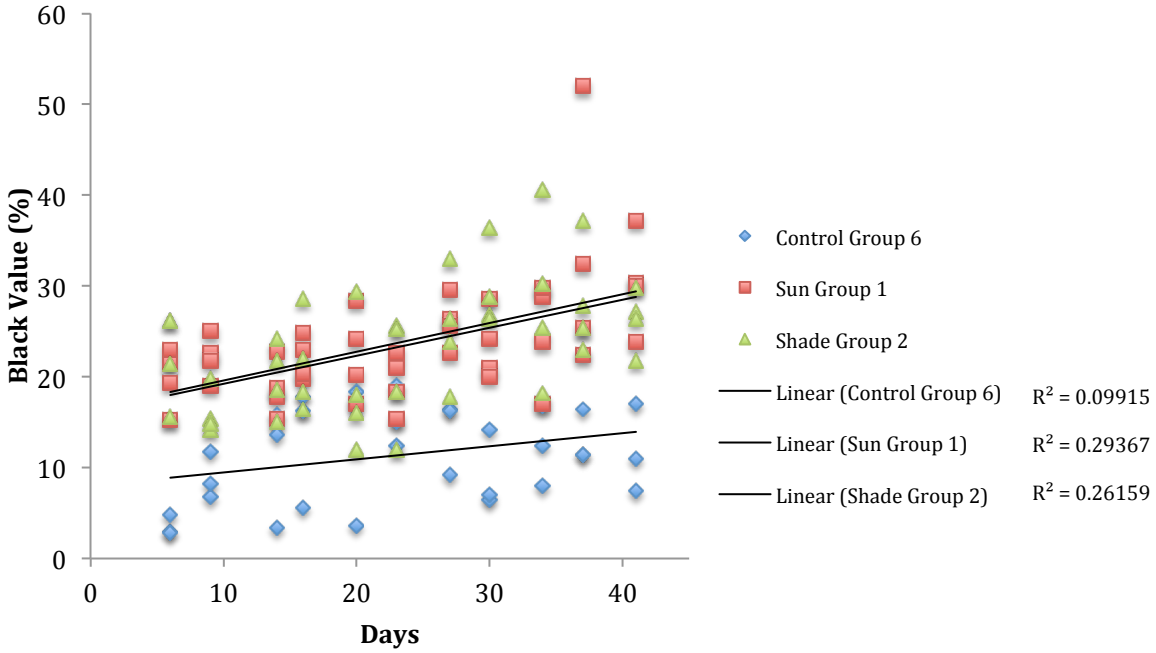

Figure 3.12: Black Values from Photos of Control Group 6, Sun Group 1, and Shade group 2 Over Time

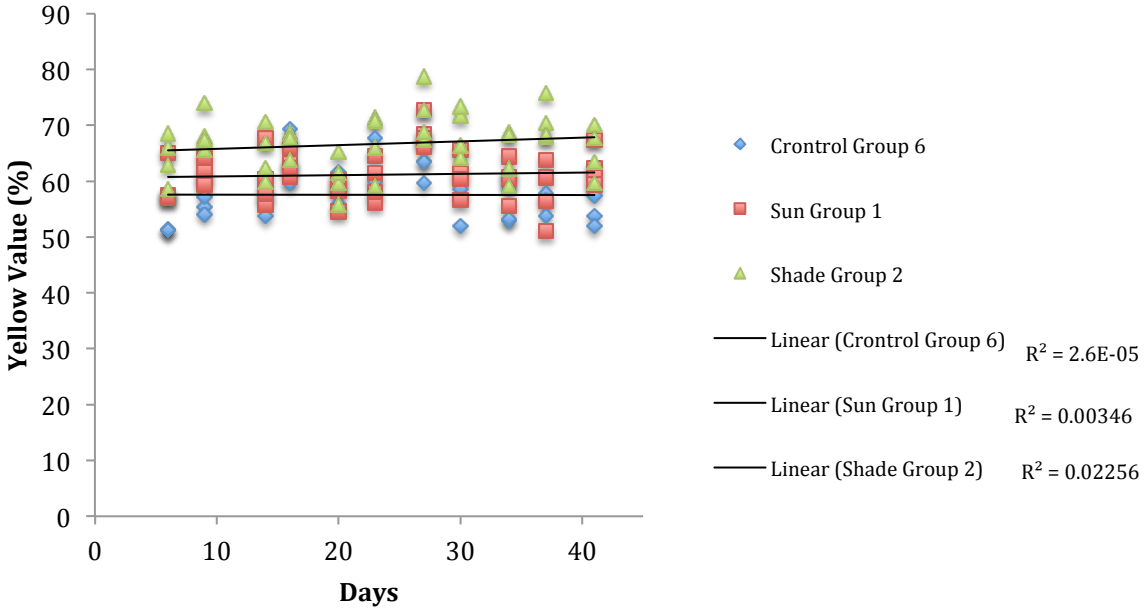

Figure 3.13: Yellow Values from Photos of Control Group 6, Sun Group 1, and Shade group 2 Over Time

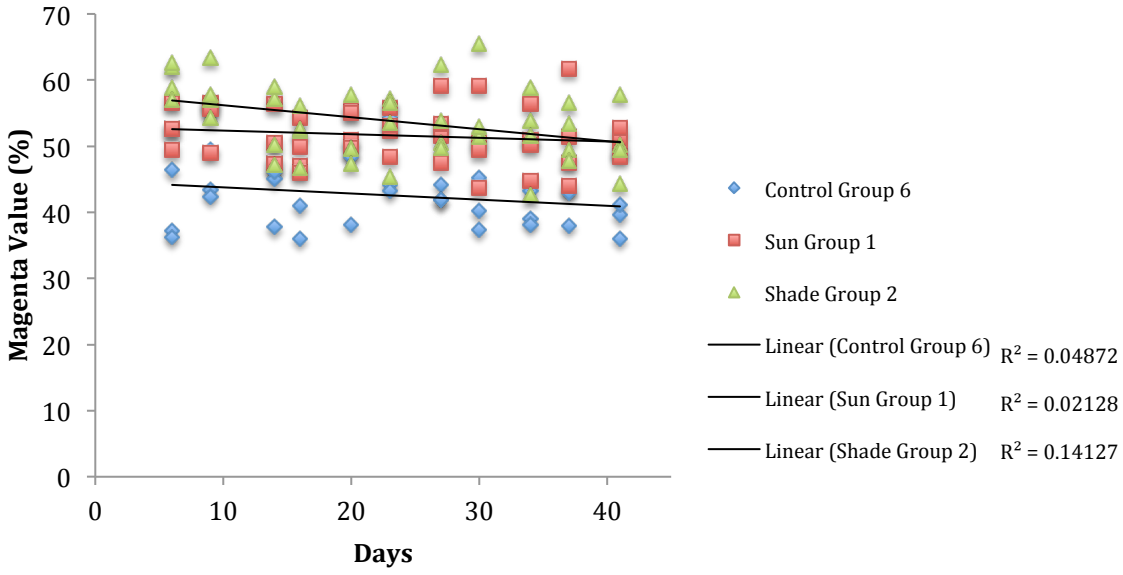

Figure 3.14: Magenta Values from Photos of Control Group 6, Sun Group 1, and Shade group 2 Over Time

3.2.2 Munsell® Colour Results Before and After Maceration

The other method of colour quantification used involved assigning Munsell Soil Colour Chart (MSCC) nominal values to every specimen both before and after maceration. Many bones experienced mould growth and likely other bacterial growth, as well as staining likely due to lipid retention (17). As a result, there appeared to be a great deal of colour fluctuation throughout the 43-day exposure period. One representative specimen of this phenomenon, specimen 16, was photographed along side its two corresponding MSCC's during its colour assessment and macroscopic analysis pre-maceration (Figure 3.15). A variety of colours are evident within the area of analysis (delineated by the paper square) likely due to bacterial growth or bone staining from lipid retention.

Specimen colours were sometimes found to vary day-to-day, depending on the precipitation. For instance, on days where it rained and the bones were photographed, it is apparent in the images that they took on a more waxy pale appearance in comparison to the resulting photos of the same specimens from a day without precipitation (Figure 3.16). Due to the vast array of fluctuating colours observed in some bones' area of analysis, more than one Munsell Soil Colour was assigned per bone. The results of the Munsell Colour Chart analysis are summarized in Table 3.2 and Table 3.3. Generally, prior to maceration, the bones exhibited a higher degree of variability in colour with varying degrees of red, pink, and purple tinges on specimens. This was likely due to remaining soft tissue on the bone surface that had undergone decomposition, as well as marrow staining. After maceration, no matter what the group, the bones took on a more yellow/light beige

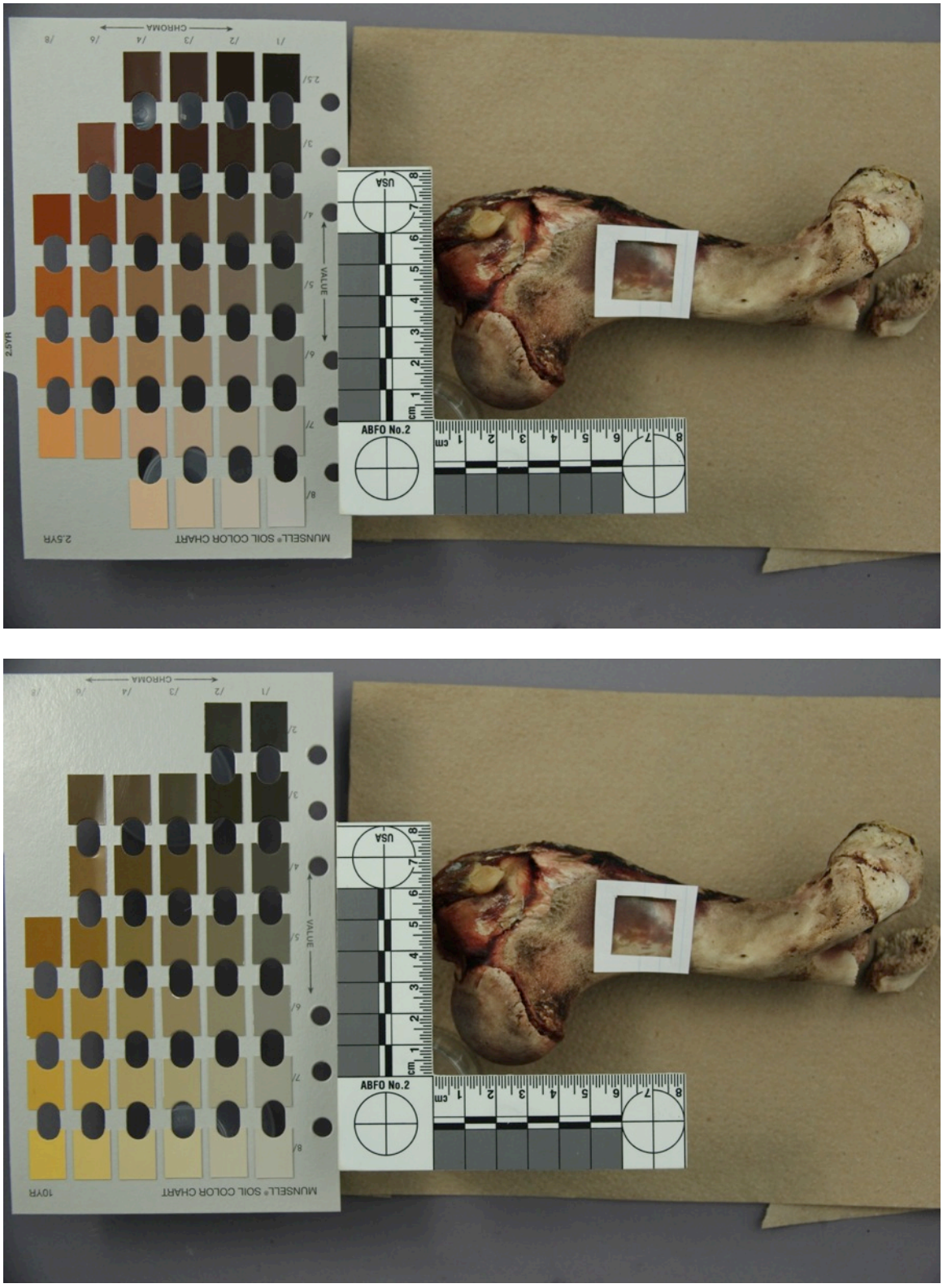

Figure 3.15: Specimen 16 along side its two corresponding Munsell Soil Charts during its colour assessment and macroscopic analysis, pre-maceration

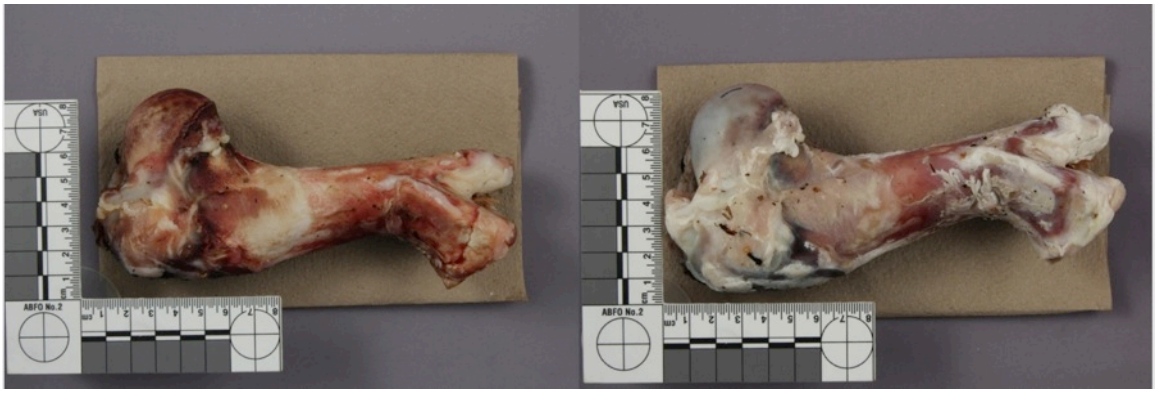

Figure 3.16: Comparison of specimen 1 on a day without precipitation (left), and with precipitation (right)

Table 3.2: Munsell Soil Colour Chart Results for Control Specimens and Groups 3, 4, and 5

Group	Specimen	Munsell Soil Chart Colours
Control Group 6 Pre-Maceration	24	2.5Y 8/3
	22	10YR 7/3
	23	10YR 8/4, 2.5YR 4/4
Rock Substrate Group 3 Pre-Maceration	9	10YR 8/6, 10YR 7/6, 10YR 6/6
	10	10YR 8/4, 10YR 6/3
	11	10YR 5/4, 2.5YR 8/1, 2.5YR 6/1
	12	2.5Y 7/3, 2.5Y 5/3
Moist Soil Substrate Group 4 Pre-Maceration	13	2.5YR 5/1, 2.5YR 4/1
	14	2.5YR 4/2, 2.5YR 8/1
	15	2.5YR 4/1, 2.5YR 3/1
	16	2.5YR 3/1, 10YR 6/3

Grass Substrate Group 5 Pre-Maceration	17	10R 2.5/1, 10R 4/4, 2.5Y 5/4
	18	2.5Y 3/3
	19	2.5Y 5/4, 10R 3/3
	20	2.5Y 6/2, 10R 2.5/1
Control Group 6 Post-Maceration	22	2.5Y 7/3
	23	2.5Y 8/2, 2.5Y 7/2
	24	2.5Y 8/1, 2.5Y 7/2
Rock Substrate Group 3 Post-Maceration	9	2.5Y 8/3, 2.5Y 7/2
	10	2.5Y 8/2, 2.5Y 8/3, 2.5Y 7/3
	11	2.5Y 7/3, 2.5Y 5/2
	12	2.5Y 8/2, 2.5Y 7/2
Moist Soil Substrate Group 4 Post-Maceration	13	2.5Y 7/2, 2.5Y 5/1, 2.5Y 4/1
	14	2.5Y 8/2, 2.5Y 5/2, 2.5Y 2.5/1
	15	2.5Y 7/2, 2.5Y 6/2
	16	2.5Y 7/2, 2.5Y 6/2
Grass Substrate Group 5 Post-Maceration	17	2.5YR 3/1
	18	2.5Y 8/2, 2.5YR 5/2
	19	2.5Y 8/3, 2.5Y 7/3
	20	2.5Y 8/3, 2.5Y 6/1

(Table 3.2 Continued)

Table 3.3: Munsell Soil Colour Chart Results for Groups 1 and 2 Left In Winter Environment

Group	Specimen	Munsell Soil Chart Colours
Group 1 Winter Environment Pre-Maceration	1	2.5Y 7/3, 2.5Y 6/3, 5YR 5/1
	2	5YR 5/1, 5YR 4/2, 7.5YR 5/3
	3	5YR 4/3, 5YR 3/2, 7.5YR 5/2
	4	5YR 4/4, 2.5Y 7/1
Group 2 Winter Environment Pre-Maceration	5	2.5Y 7/4, 2.5Y 6/4
	6	2.5Y 8/1, 2.5Y 7/3
	7	7.5YR 4/3, 2.5Y 8/1
	8	2.5Y 8/6, 2.5Y 8/2, 5YR 4/2
Group 1 Winter Environment Post-Maceration	1	2.5Y 8/2
	2	2.5Y 8/2, 2.5Y 8/1
	3	2.5Y 8/2, 2.5Y 6/1
	4	2.5Y 8/2
Group 2 Winter Environment Post-Maceration	5	2.5Y 8/2, 2.5Y 6/2, 2.5Y 8/1
	6	2.5Y 8/2
	7	2.5Y 8/2, 2.5Y 8/1
	8	2.5Y 8/3, 2.5Y 7/4

colour, with minor variability in the darkness of each bone. No strong trends were visible that could attribute specimens to a specific group from this analysis, except for the control group being the lightest prior to maceration.

3.2.3 Seriation of Bones Based on Colour of Area of Analysis

In addition to the Munsell Soil Colour Chart and CMYK analysis, a seriation (Figure 3.17) was performed of all the bones based on overall darkness of the area of analysis. The results of this seriation are summarized in Table 3.4. This was performed to provide a comparative basis for the bones on an ordinal scale. The seriation spans the darkest observed area of analysis to the lightest. Those that cannot be significantly visually separated are placed within the same delineated row by lines. Bones of the same groups are colour coded, the control group 6 being blue, the sun/winter environment group 1 being green, the shade/winter environment group 2 being orange, the rock substrate group 3 being black, the moist soil substrate group 4 being purple, and the grass substrate group 5 being gray for easier visual comparison. As can be noted, some of the darkest and lightest bones are both from the control group (blue) that experienced no weathering. The two groups that experienced the most weathering being the sun/winter environment group and the shade/winter environment group (green and orange) experience similar colour changes since they are all next to one another in the Seriation. It should also be noted that specimen “14” of the moist soil group was scavenged and replaced with control bone 21 after roughly a week of environmental exposure passed, so it may not be unusual that it is found further away than the other bones

Figure 3.17: Seriation of all specimens

Table 3.4: Summarization of Seriation of Figure 3.12.

Seriation Number	Specimen
1	17
2	23
3	21<->14 *
4	20
5	18
6	11
7	13
8	19
9	16
10	15
11	8
12	6
13	3
14	1
15	7
16	4
17	2
18	5
19	10
20	9
21	12
22	22
23	24

from it's group (purple). The specimens of the rock substrate (black) group were found close to one another for the most part with the exception of specimen 11, and those of the grass substrate group 5 (gray) seemed to experience some variety of colour from one another in their AOA seeing as they are not all closely found beside one another.

3.3 Bone Macroscopic Analysis

3.3.1 Visual Macroscopic Results Before and After Maceration

Not only was a macroscopic analysis conducted of every specimen with a dissection microscope, but photos were also taken with a macro lens of all specimens post-maceration. Evidence of taphonomic change was visible on the specimens both before and after maceration, with a few exhibiting flaking/exfoliation, cracking, and diminished coverage of periosteum. One exemplary specimen with visible reduction of periosteum within the area of analysis is specimen 19, and it was photographed along side its corresponding MSCC during the pre-maceration colour assessment (Figure 3.18). Another exemplary specimen that exhibited visible cracking, exfoliation, and expected lack of periosteum post-maceration is specimen 16 (Figure 3.19). All specimens from the moist soil group that were submerged in water on more that one occasion underwent epiphyseal separation and significant loss of any remaining soft tissue, even before maceration. Many other specimens also showed signs of taphonomic change, and were photographed accordingly. The results of the macroscopic analysis are further summarized in Tables 3.5 to 3.8.

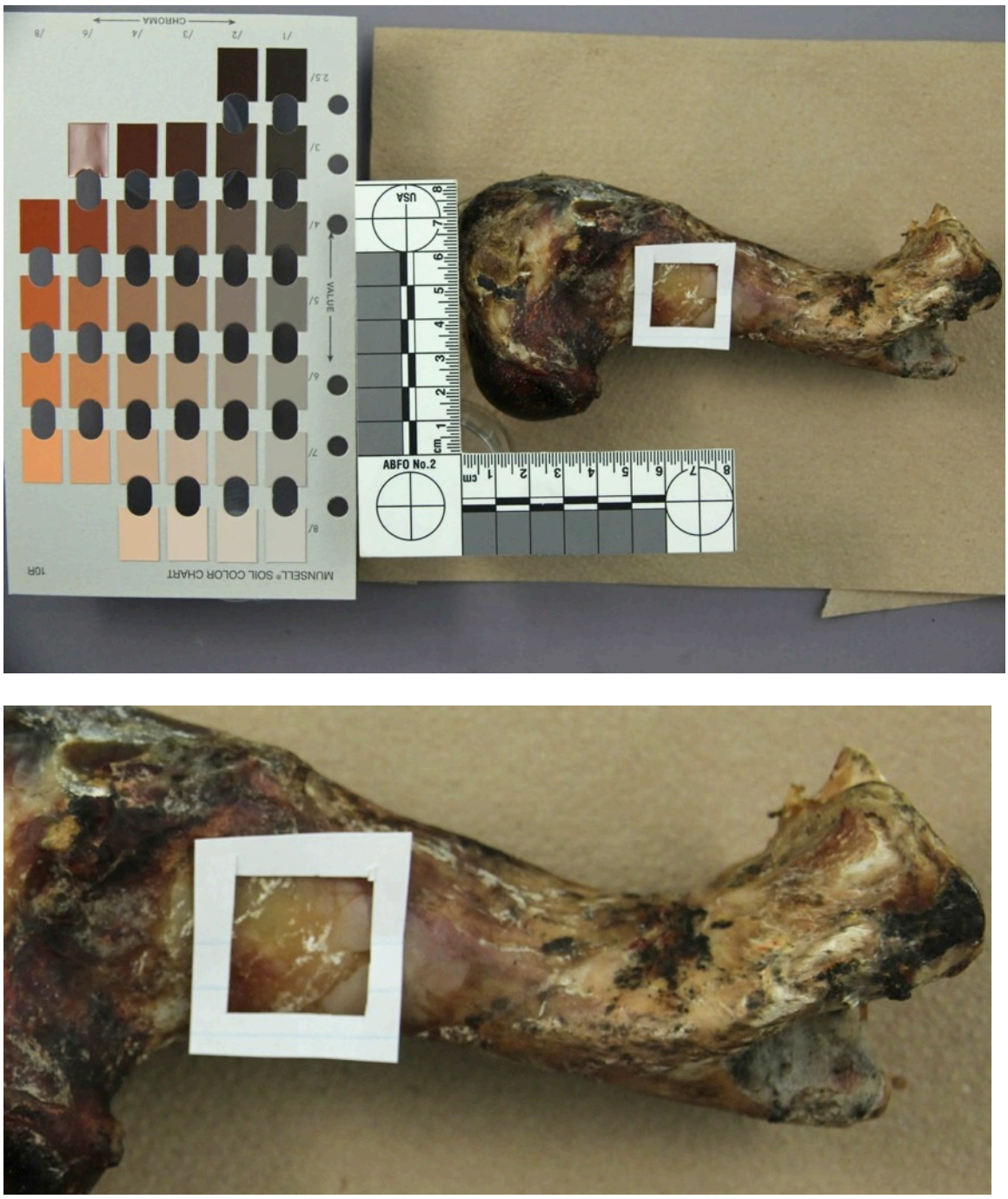

Figure 3.18: Visible reduction of periosteum in area of analysis (within paper square).

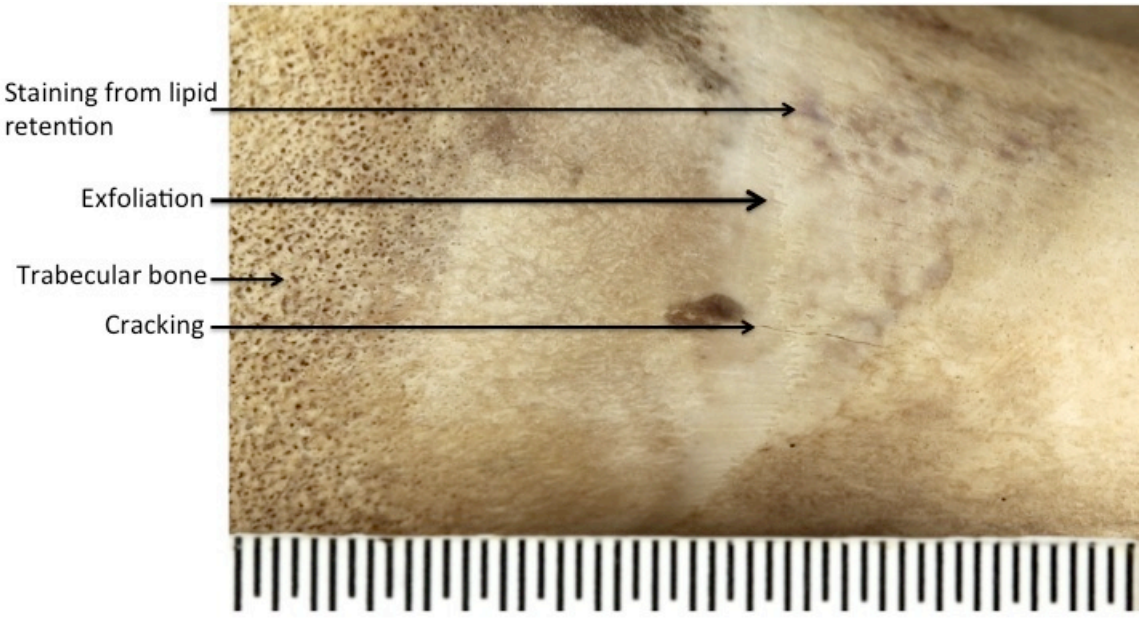

Figure 3.19: Visible cracking, exfoliation, and expected lack of periosteum post-maceration of specimen 16.

Table 3.5: Macroscopic Analysis of Substrate Groups 3, 4, 5, and control group 6 Pre-Maceration

Specimen	Presence of Cracking	# Visible Cracks	Presence of Flaking	> Or < 50%	Presence of Periosteum	> Or < 50%
24	No	N/A	Yes	<50%	Yes	>50%
22	No	N/A	Yes	<50%	Yes	>50%
23	No	N/A	Yes	<50%	Yes	>50%
9	No	N/A	Yes	<50%	Yes	>50%
10	No	N/A	No	N/A	Yes	>50%
11	No	N/A	No	N/A	Yes	>50%
12	No	N/A	No	N/A	Yes	>50%
13	No	N/A	No	N/A	Yes	<50%
14	No	N/A	Yes	<50%	Yes	<50%
15	No	N/A	No	N/A	No	N/A
16	Yes	4	Yes	<50%	No	N/A
17	No	N/A	Yes	<50%	Yes	>50%
18	No	N/A	No	N/A	Yes	>50%
19	No	N/A	Yes	<50%	Yes	>50%
20	No	N/A	Yes	>50%	Yes	>50%

Table 3.6: Macroscopic Analysis of Substrate Groups 3, 4, 5, and control group 6 Post-Maceration

Specimen	Presence of Cracking	# Visible Cracks	Presence of Flaking	> Or < 50%	Presence of Periosteum	> Or < 50%
22	No	N/A	No	N/A	No	N/A
23	No	N/A	Yes	>50%	No	N/A
24	No	N/A	no	N/A	No	N/A
9	No	N/A	Yes	<50%	No	N/A
10	No	N/A	Yes	<50%	No	N/A
11	No	N/A	Yes	<50%	No	N/A
12	No	N/A	Yes	<50%	No	N/A
13	Not in AOA	N/A	Yes	>50%	No	N/A
14	Not in AOA	N/A	Yes	>50%	No	N/A
15	Not in AOA	N/A	Yes	>50%	No	N/A
16	Yes	1	Yes	>50%	No	N/A
17	No	N/A	Yes	>50%	No	N/A
18	Not in AOA	N/A	Yes	>50%	No	N/A
19	No	N/A	Yes	<50%	No	N/A
20	No	N/A	Yes	>50%	No	N/A

Table 3.7: Macroscopic Analysis of Sun and Shade Groups 1 and 2 Pre-Maceration

Specimen	Presence of Cracking	# Visible Cracks	Presence of Flaking	> Or < 50%	Presence of Periosteum	> Or < 50%
1	No	N/A	No	N/A	Yes	>50%
2	No	N/A	No	N/A	Yes	>50%
3	No	N/A	No	N/A	Yes	>50%
4	No	N/A	No	N/A	Yes	>50%
5	No	N/A	No	N/A	Yes	>50%
6	No	N/A	Yes	<50%	Yes	<50%
7	No	N/A	Yes	<50%	Yes	>50%
8	No	N/A	Yes	<50%	Yes	>50%

Table 3.8: Macroscopic Analysis of Sun and Shade Groups 1 and 2 Post-Maceration

Specimen	Presence of Cracking	# Visible Cracks	Presence of Flaking	> Or < 50%	Presence of Periosteum	> Or < 50%
1	No	N/A	Not in AOA	N/A	No	N/A
2	No	N/A	Not in AOA	N/A	No	N/A
3	Not in AOA	N/A	Not in AOA	N/A	No	N/A
4	Not in AOA	N/A	Not in AOA	N/A	No	N/A
5	No	N/A	No	N/A	No	N/A
6	No	N/A	Not in AOA	N/A	No	N/A
7	Not in AOA	N/A	Not in AOA	N/A	No	N/A
8	Not in AOA	N/A	Not in AOA	N/A	No	N/A

3.3.2 Chi Square Results of Features Observed

Chi square tests were performed to determine whether the presence or absence of cracking, flaking, and periosteum were independent or dependant of the group from which the specimen came, both before and after maceration. The results are summarized in table 3.9. The only two tests out of the six performed that provided significant differences between expected and observed numbers were for the presence and absence of cracks post-maceration, and for the presence and absence of flaking pre-maceration. Groups 1, 2, and 4 exhibited more presence of cracking post-maceration in the observed counts than the expected counts, and the remaining groups 3, 5, and 6 showed less in the observed counts than the expected counts. Groups 2, 5, and 6 exhibited more presence of flaking pre-maceration in the observed counts than the expected counts, and groups 1, 3 and 4 showed less in the observed counts than the expected counts.

3.4 Bone Microscopic Analysis

3.4.1 Features Observed for Chosen Specimens With SEM Analysis

As previously mentioned, some specimens exhibited mould growth and bacterial growth. In order to evaluate the specific effects of bioerosion, scanning electron microscopy is required (4), and was conducted subsequent to the macroscopic analysis. Rather than conducting 23 SEM analyses, 8 specimens were chosen that were either a representative specimen of their group, or because they appeared to exhibit the most interesting features. A summary of the specimens chosen and the reasoning behind that choice can be found in table 3.10. Apparent

Table 3.9: Chi square results for all six tests where α=0.05. Bolded values were from tests that found groups to be statistically different.

Test	ρ value obtained
Absence or Presence of Cracks Pre-Maceration	0.420
Absence or Presence of Cracks Post-Maceration	**0.043**
Absence or Presence of Flaking Pre-Maceration	**0.036**
Absence or Presence of Flaking Post-Maceration	0.062
Absence or Presence of Periosteum Pre-Maceration	0.065
Absence or Presence of Periosteum Post-Maceration	Invalid results; there was no presence of periosteum in any specimen.

Table 3.10: Summary of specimens chosen for SEM analysis

Specimen Chosen	Reason
4	Representative of group 1. Suspected mould and bacterial growth.
7	Representative of group 2. Suspected mould and bacterial growth.
12	Representative of group 3. Suspected mould and bacterial growth.
16	Representative of group 4 and had crack in AOA. Suspected mould and bacterial growth.
17	Darkest bone of all. Suspected mould and bacterial growth.
18	Suspected mould and bacterial growth, visible exfoliation.
23	Significant flaking and somewhat representative of control group 6.
24	Palest colour of all specimens and somewhat representative of control group 6

differences in surface microstructure and evidence of both cracking and exfoliation were examined using the resulting micrographs. Figures 3.20 to 3.27 consist of images of 1mm to 2mm scans of each specimen in order to provide a representable image of their surface microstructures.

Specimens 4 and 7 experienced the highest degree of weathering. They were left out in the winter environment 11 weeks after the other specimens were brought in. They, along with specimen 16, have a scaly textured appearance to their surfaces (Figures 3.28 to 3.30), and in some regions are very porous. These pores are transverse (Volkmans) canals that supply nutrients to the bone, and typically occur more often in juvenile bones, such as the specimens used for this study. These bones, along with specimen 16 from the moist soil substrate group (which, arguably underwent the second highest degree of weathering having been submerged in water on more than one occasion) also have the highest amount of surface fractures (Figures 3.31 to 3.36), and fractures within canals (Figures 3.37 and 3.38). Specimen 12 from the rock substrate group, and 17 from the grass substrate group both appear to have minor flaking on their surfaces (Figure 3.39). They have far less surface micro- fractures than the first specimens mentioned, but still exhibit some fractures in their superficial layer. Additionally, some regions have a bulbous texture due to lipid congealing, and so do specimens 18 (grass substrate group), 23, and 24 (both from control group), usually in openings of the bone surface (Figures 3.40 to 3.42). Specimens 18 and 23 demonstrated a great degree of exfoliation on their still remaining superficial periosteum layers (Figure 3.43 and 3.44), and also had holes forming through this layer of tissue (Figure 3.45). The remainder of their

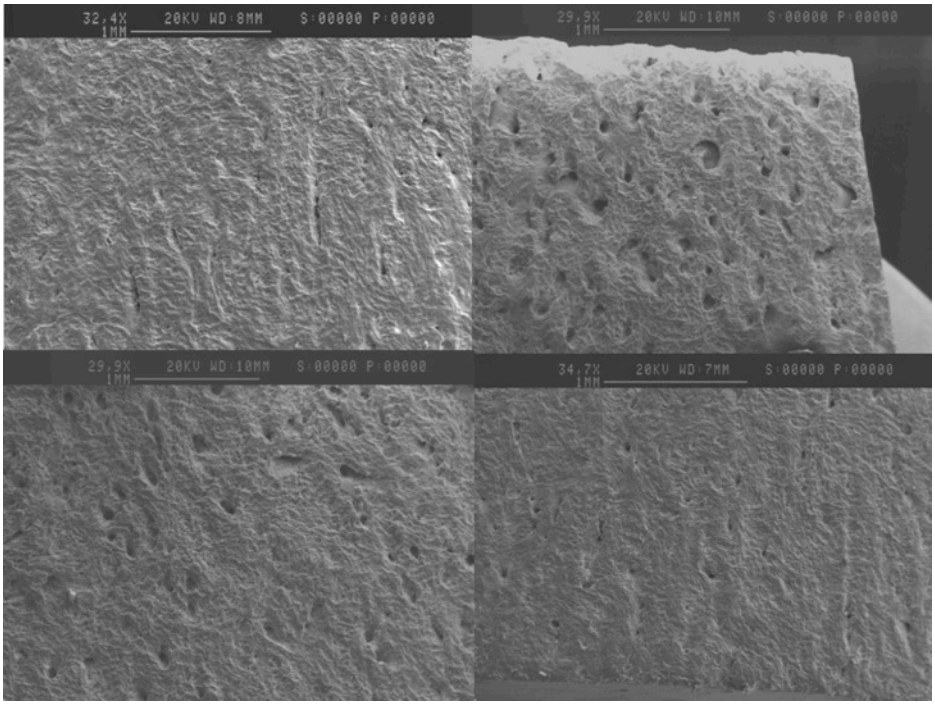

Figure 3.20: Representative images of specimen 4 surface microstructure.

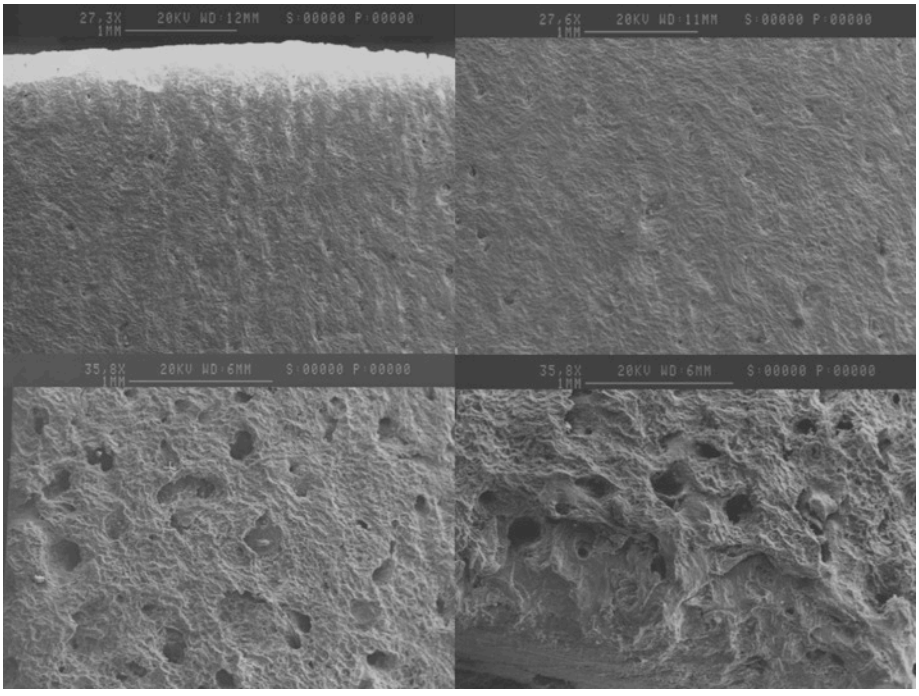

Figure 3.21: Representative images of specimen 7 surface microstructure.

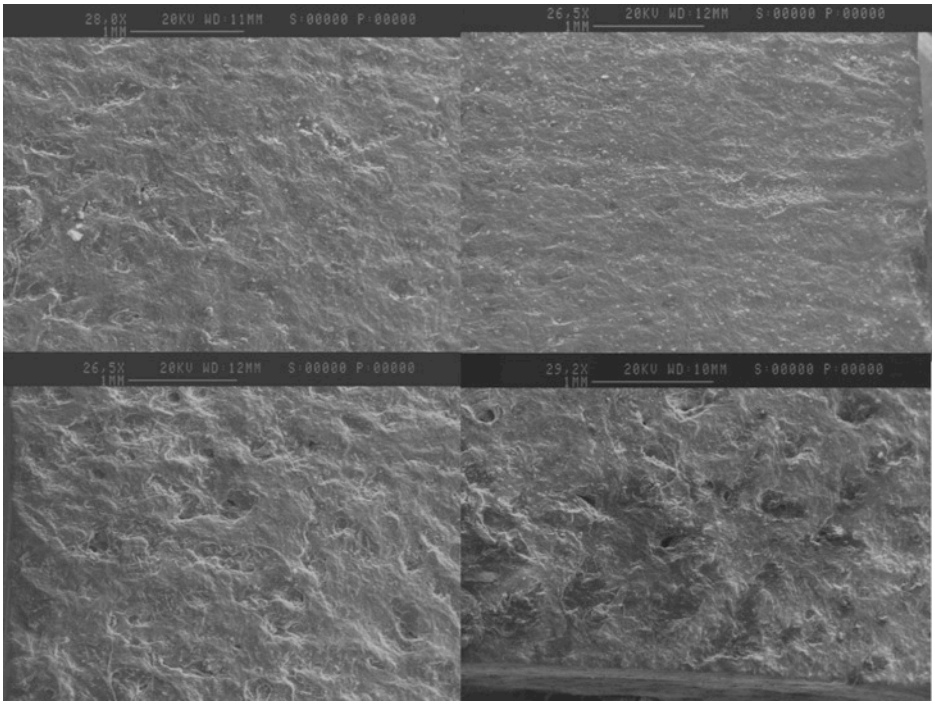

Figure 3.22: Representative images of specimen 12 surface microstructure.

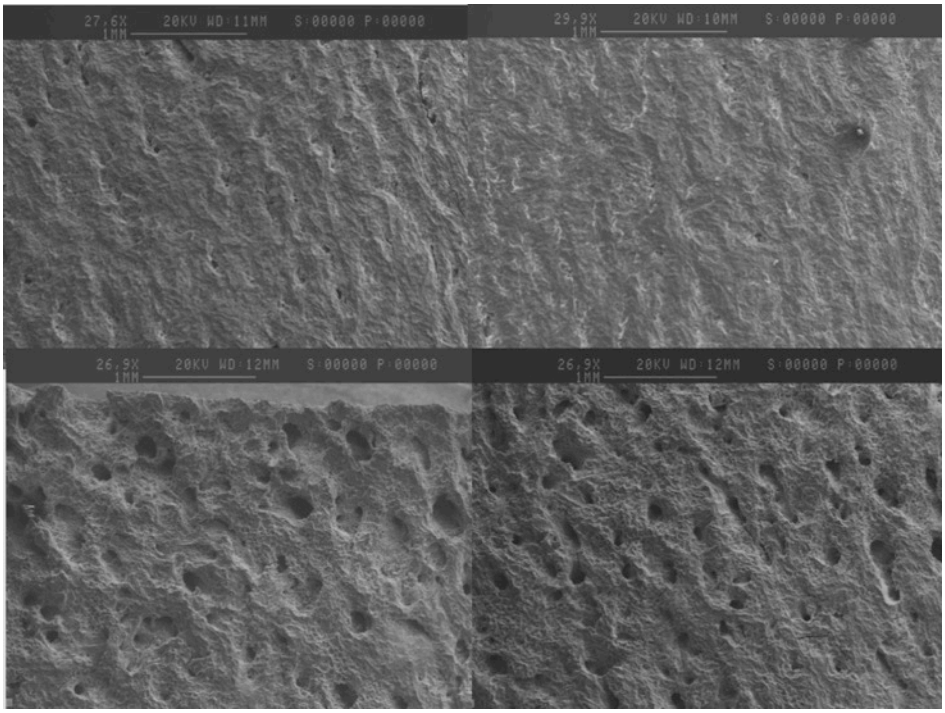

Figure 3.23: Representative images of specimen 16 surface microstructure.

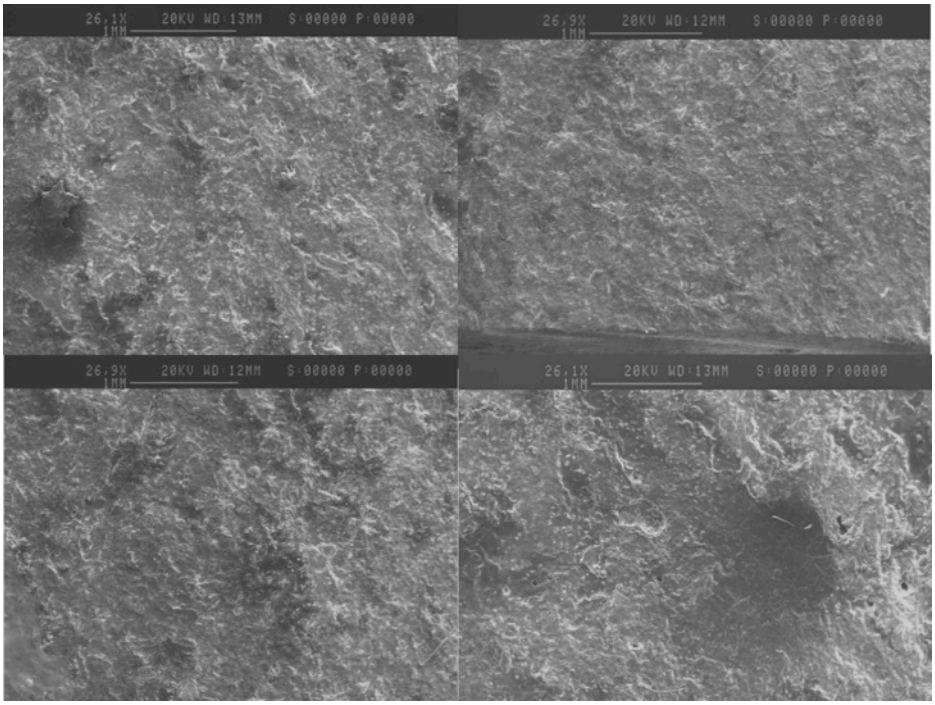

Figure 3.24: Representative images of specimen 17 surface microstructure.

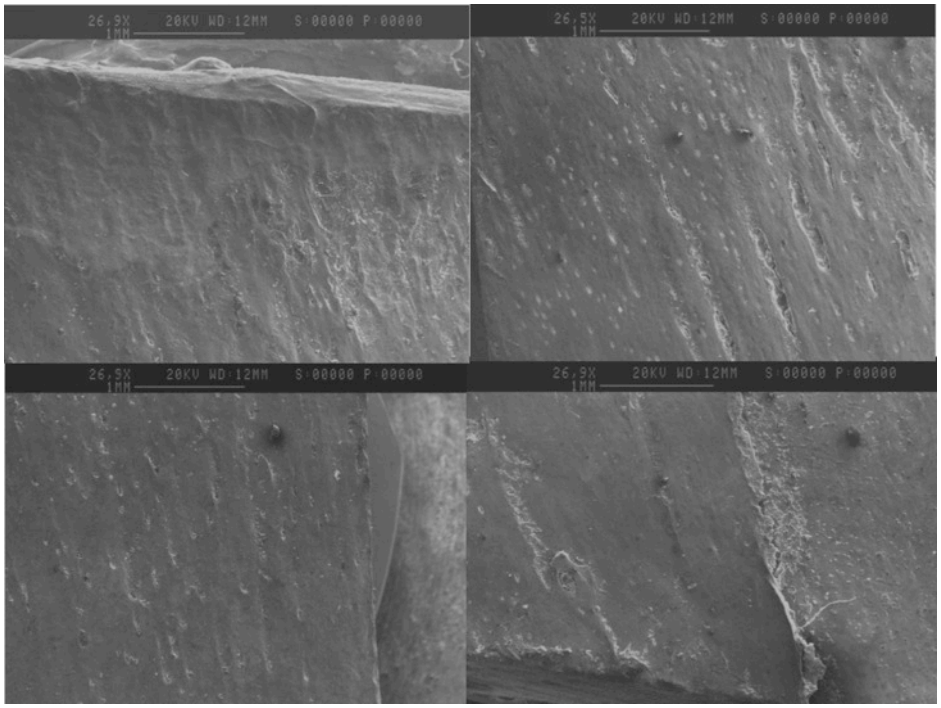

Figure 3.25: Representative images of specimen 18 surface microstructure.

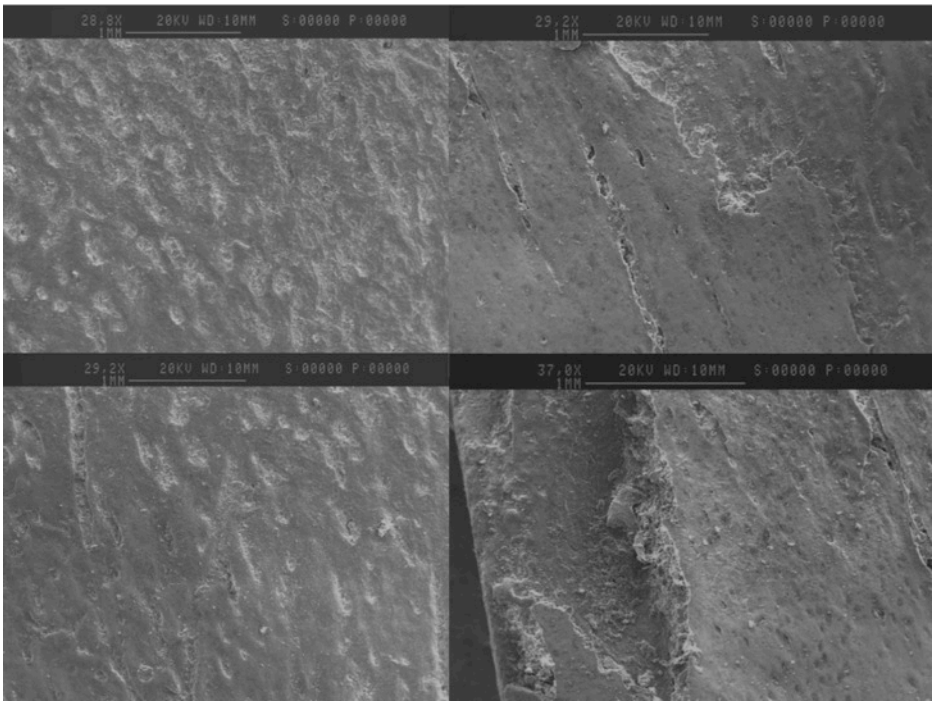

Figure 3.26: Representative images of specimen 23 surface microstructure.

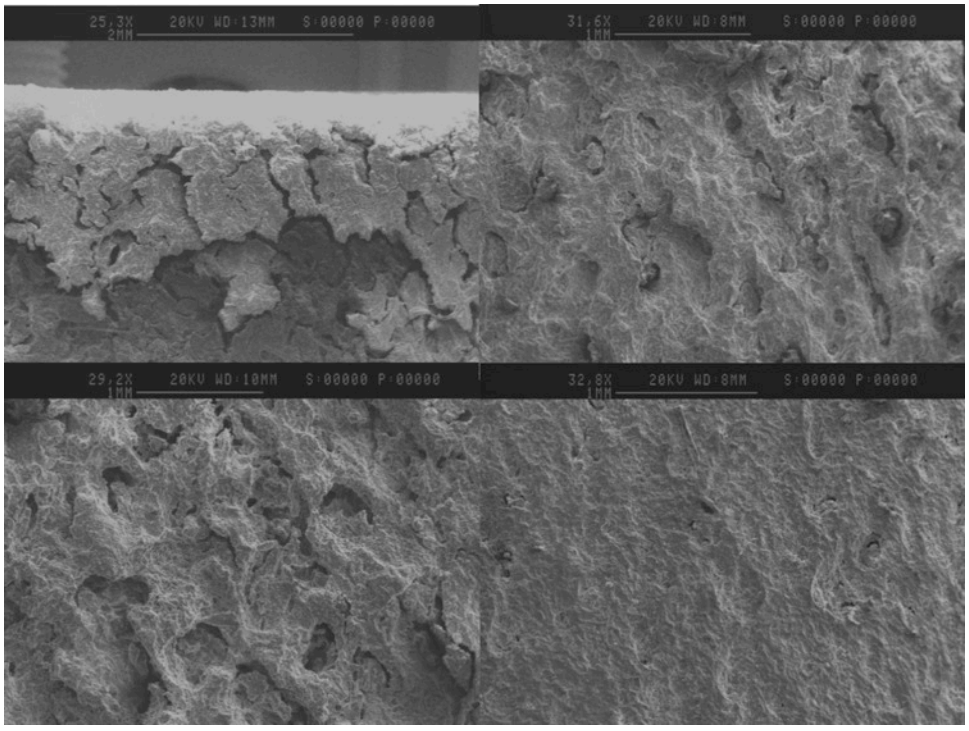

Figure 3.27: Representative images of specimen24 surface microstructure.

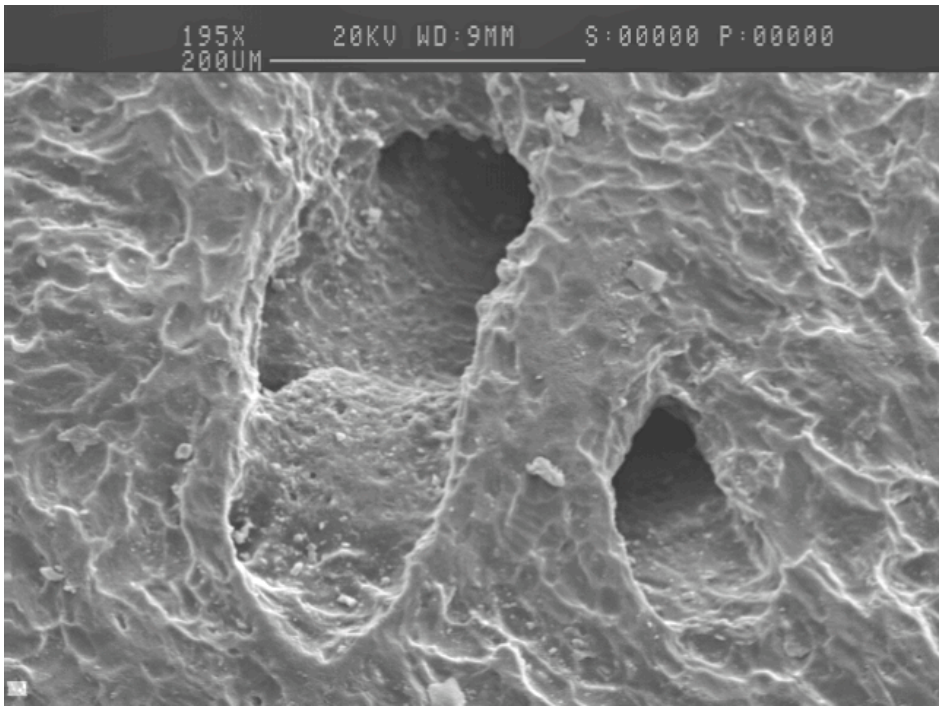

Figure 3.28: Higher magnification capturing scaly texture of specimen 4.

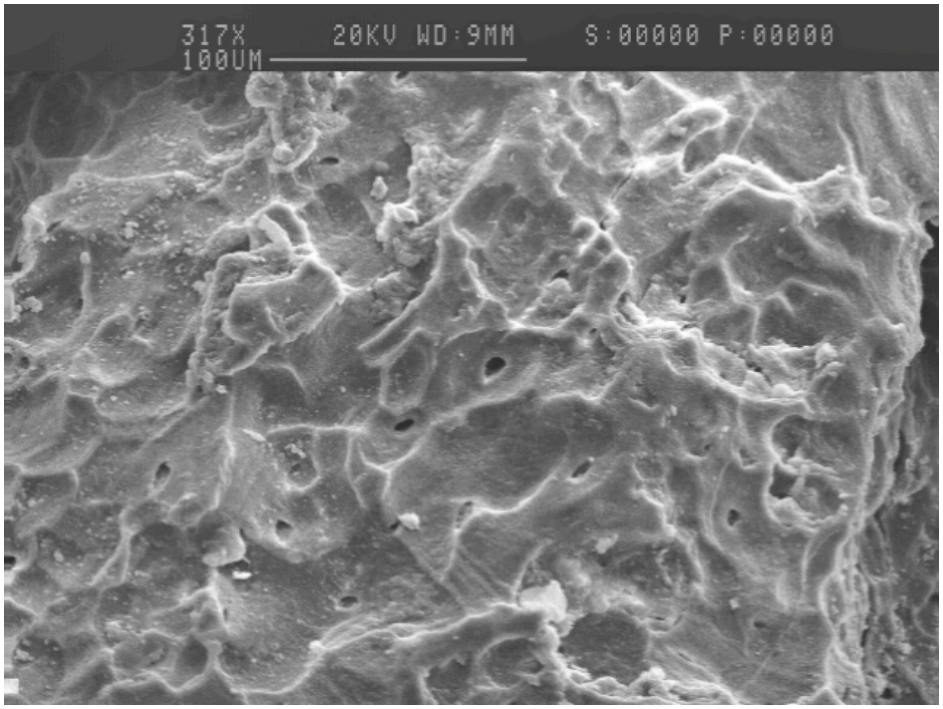

Figure 3.29: Higher magnification capturing scaly texture of specimen 7.

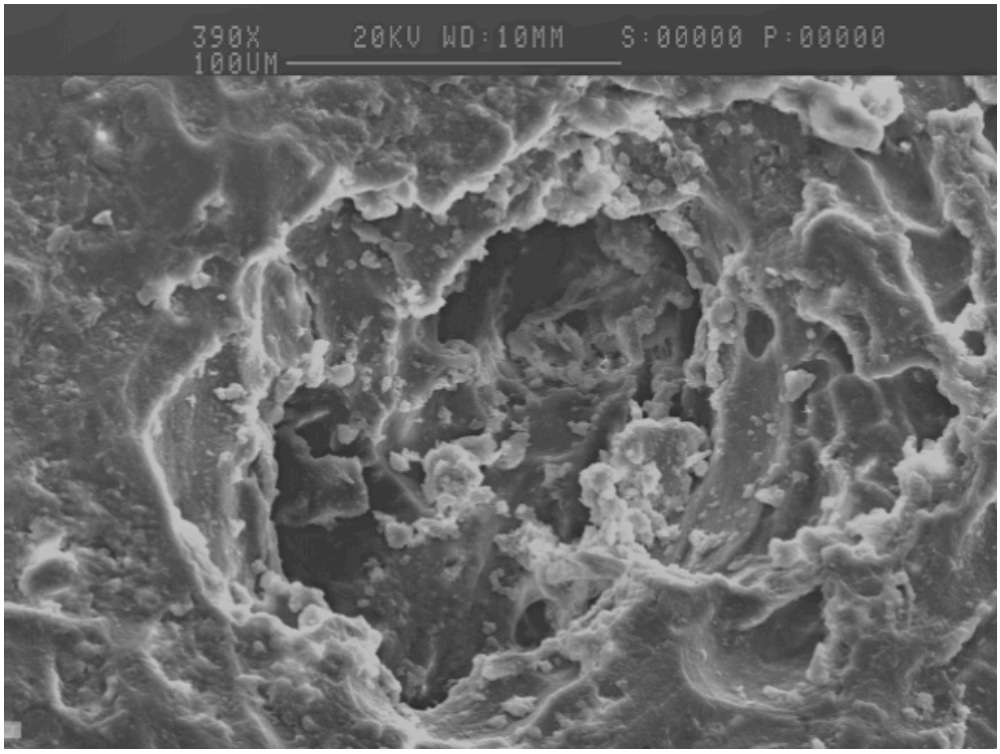

Figure 3.30: Higher magnification capturing scaly texture of specimen 16.

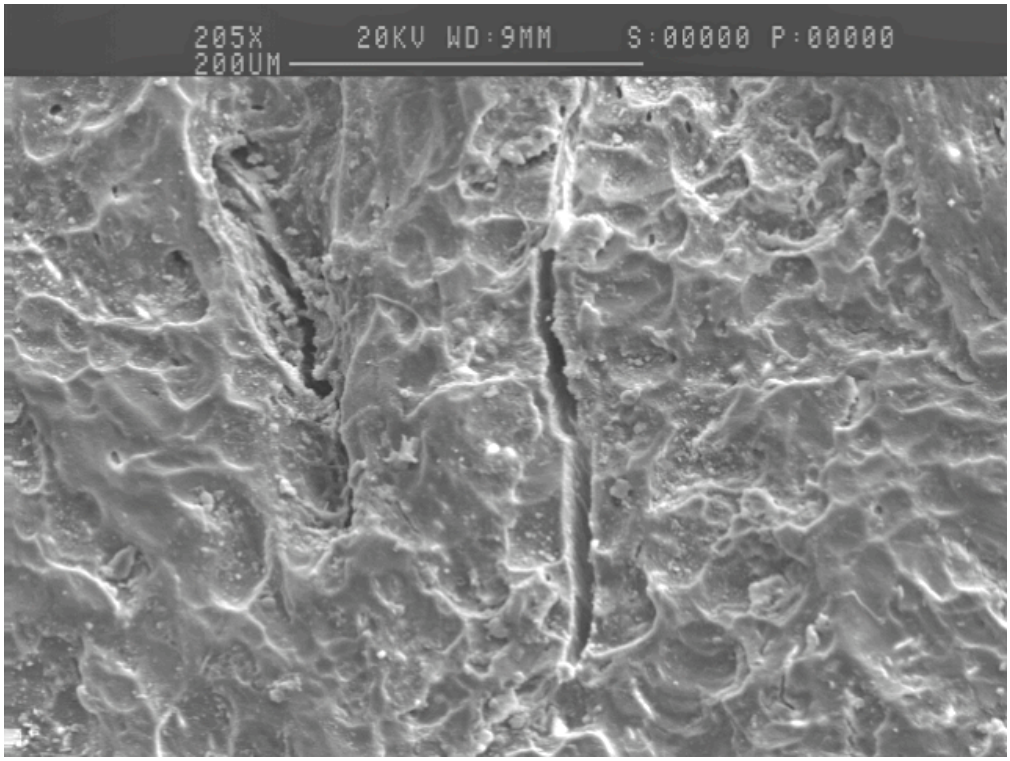

Figure 3.31: Surface microfracture of specimen 4.

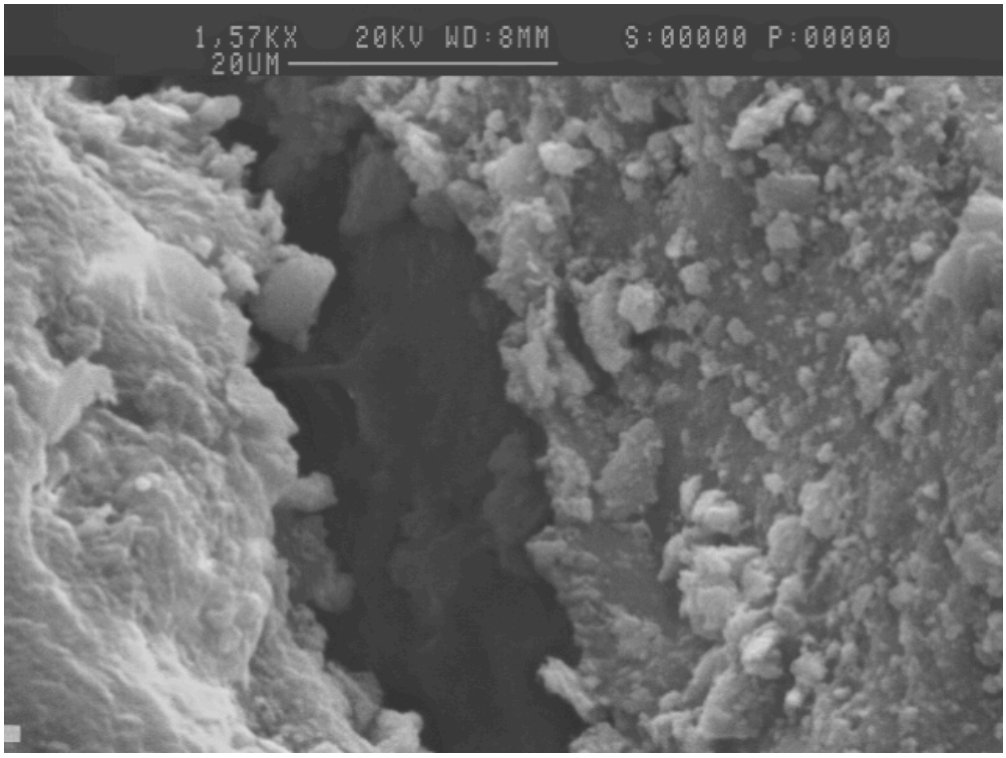

Figure 3.32: Increased magnification of a surface microfracture on specimen 4.

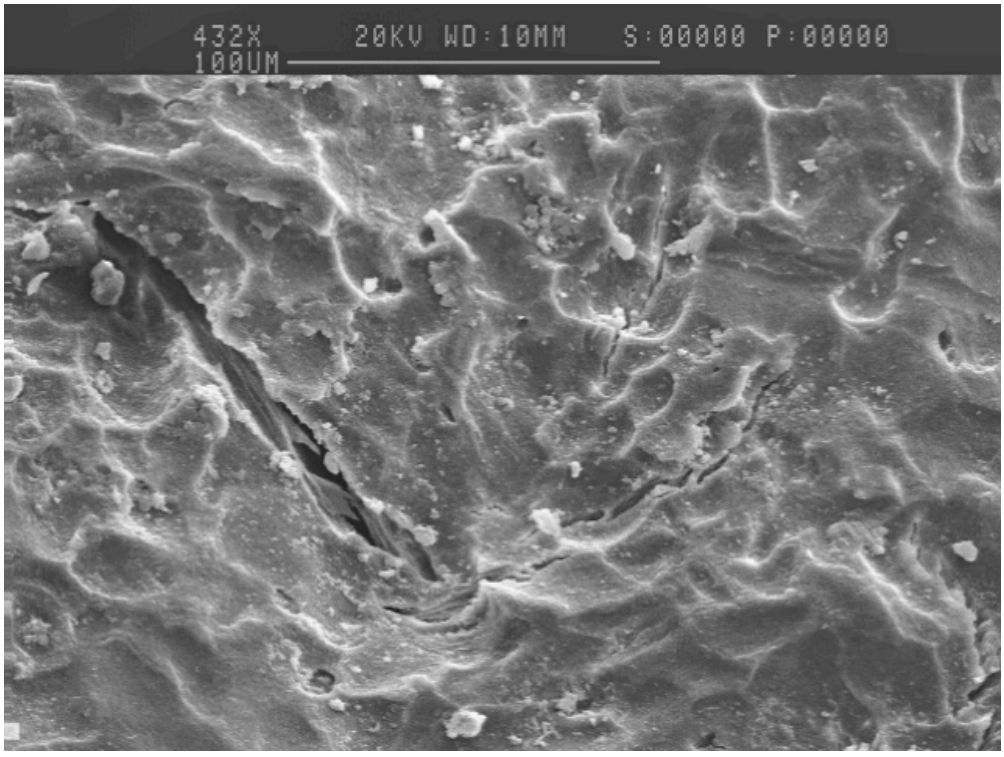

Figure 3.33: Surface microfracture of specimen 7.

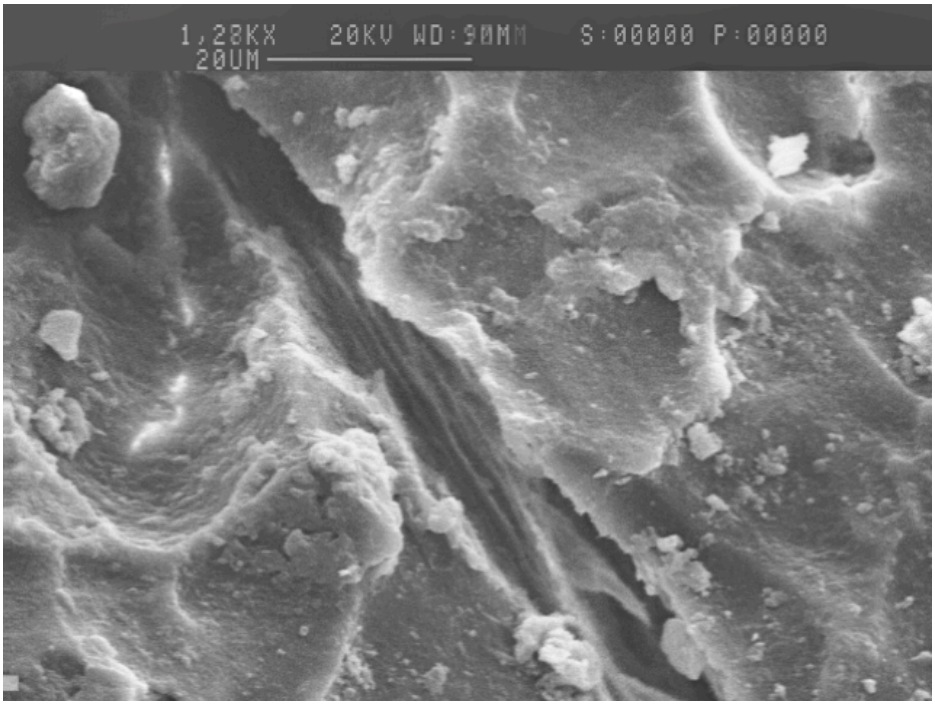

Figure 3.34: Increased magnification of surface microfracture on specimen 7.

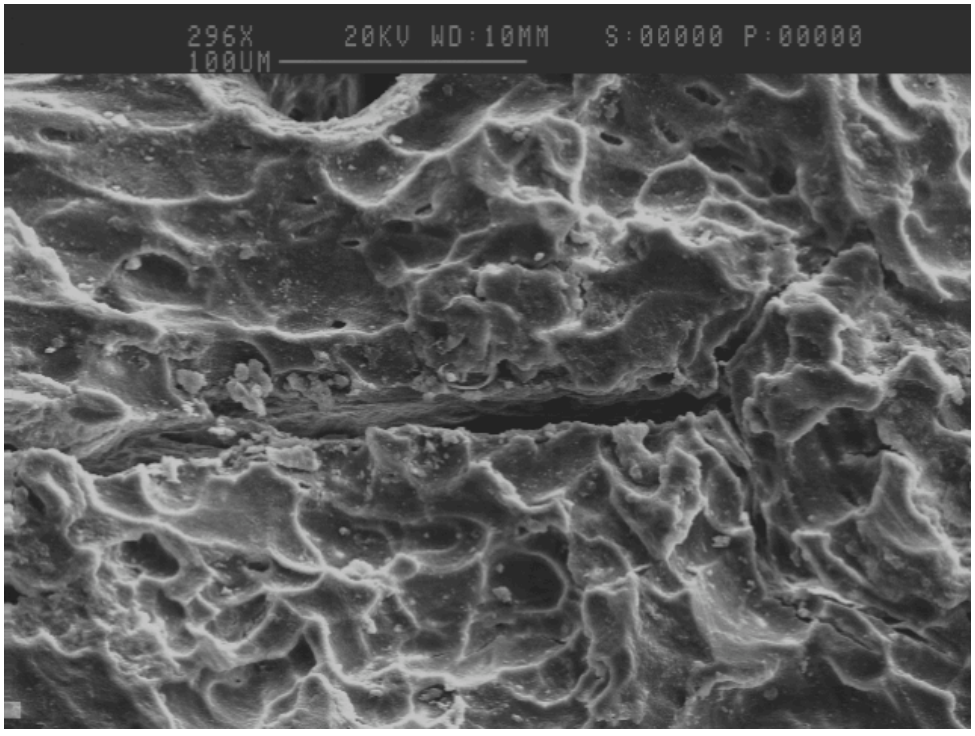

Figure 3.35: Surface microfractures of specimen 16.

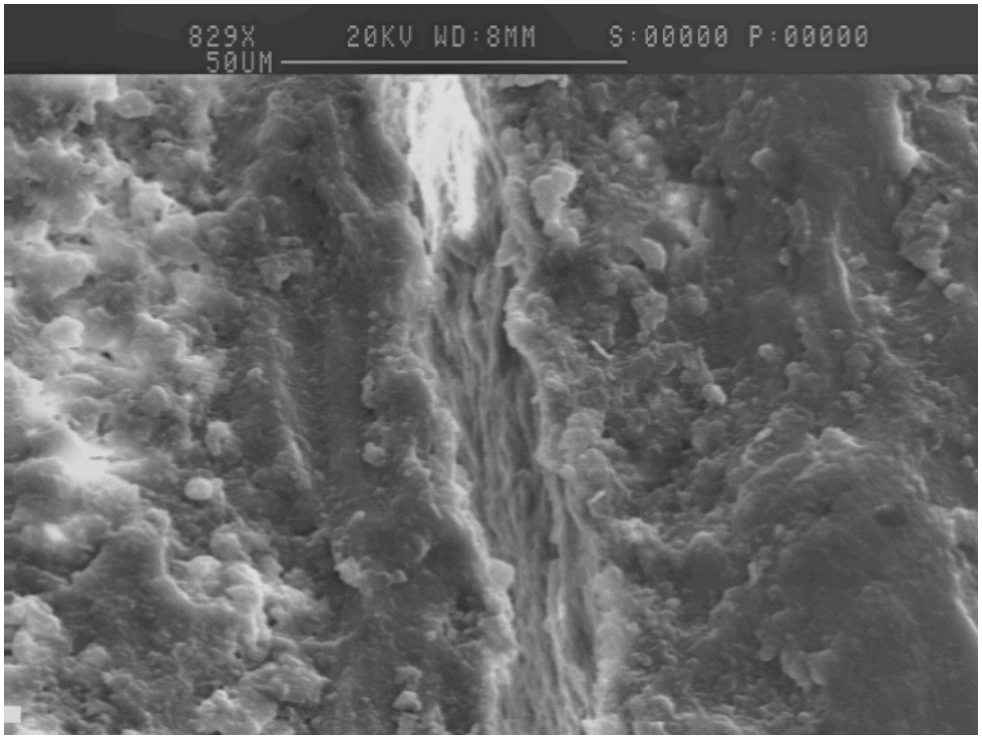

Figure 3.36: Increased magnification of a surface microfracture on specimen 16.

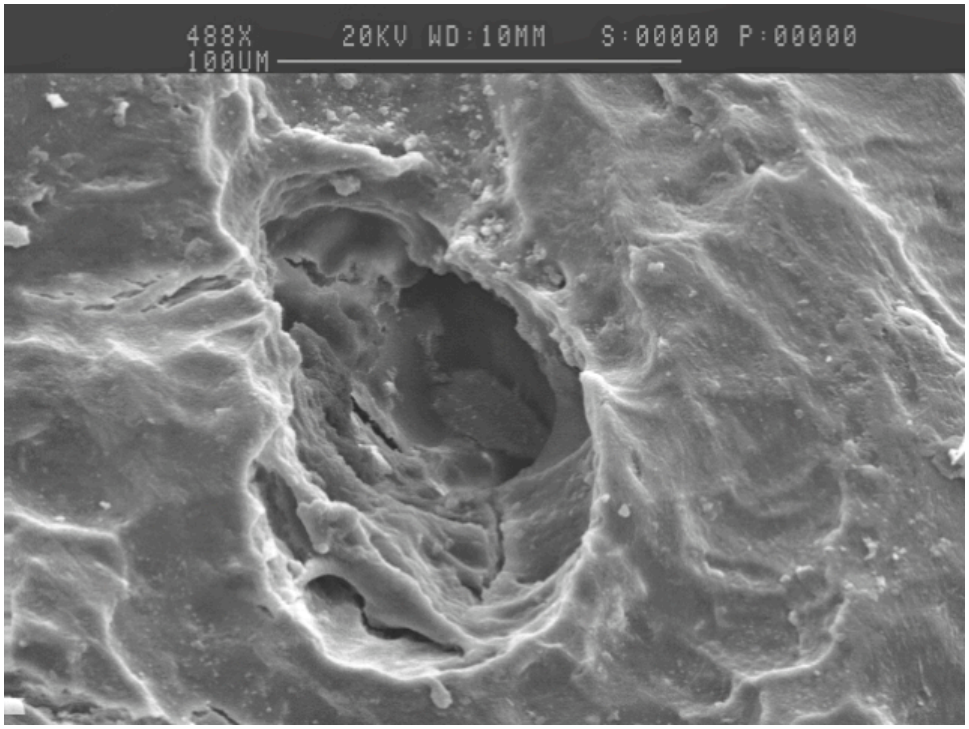

Figure 3.37: Fractured canal of specimen 4.

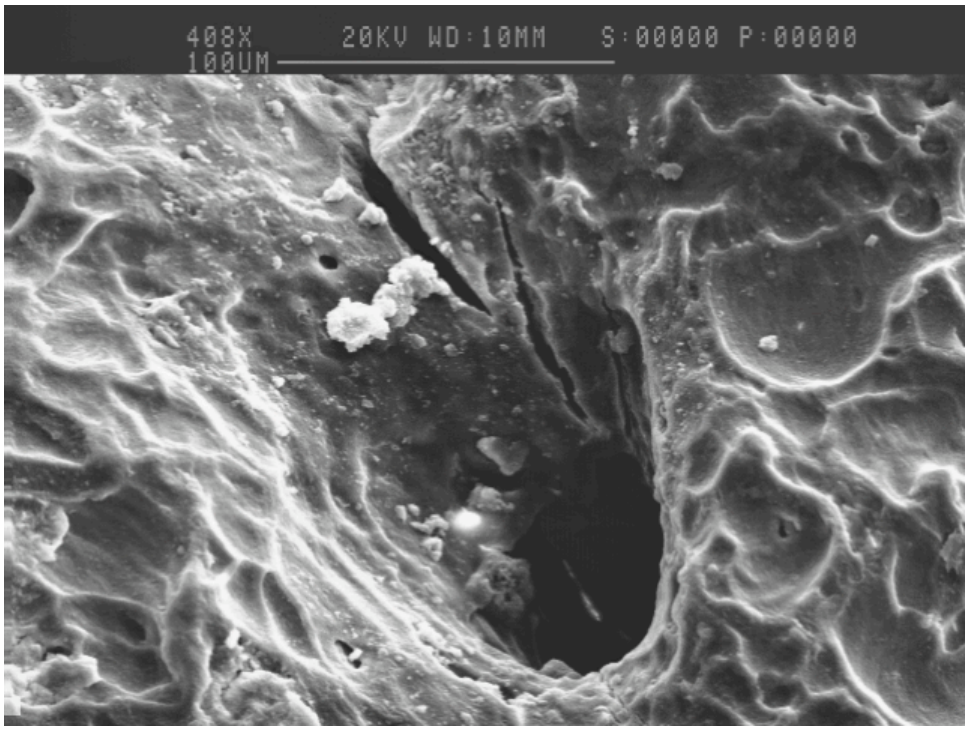

Figure 3.38: Fractured canal of specimen 16.

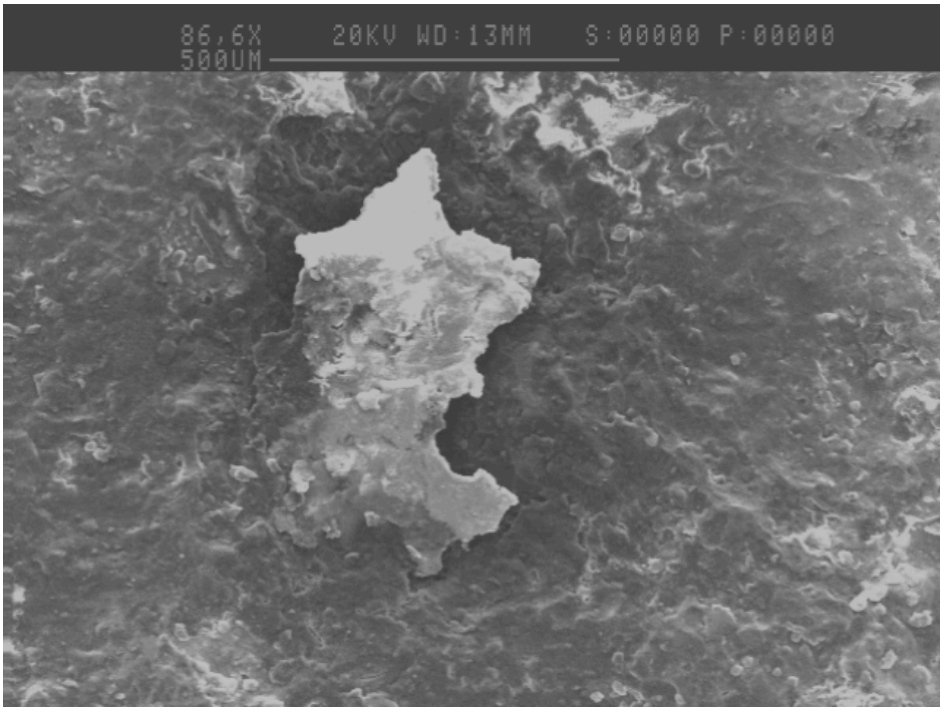

Figure 3.39: Example of minor flaking from specimen 17 (also exhibited by specimen 12).

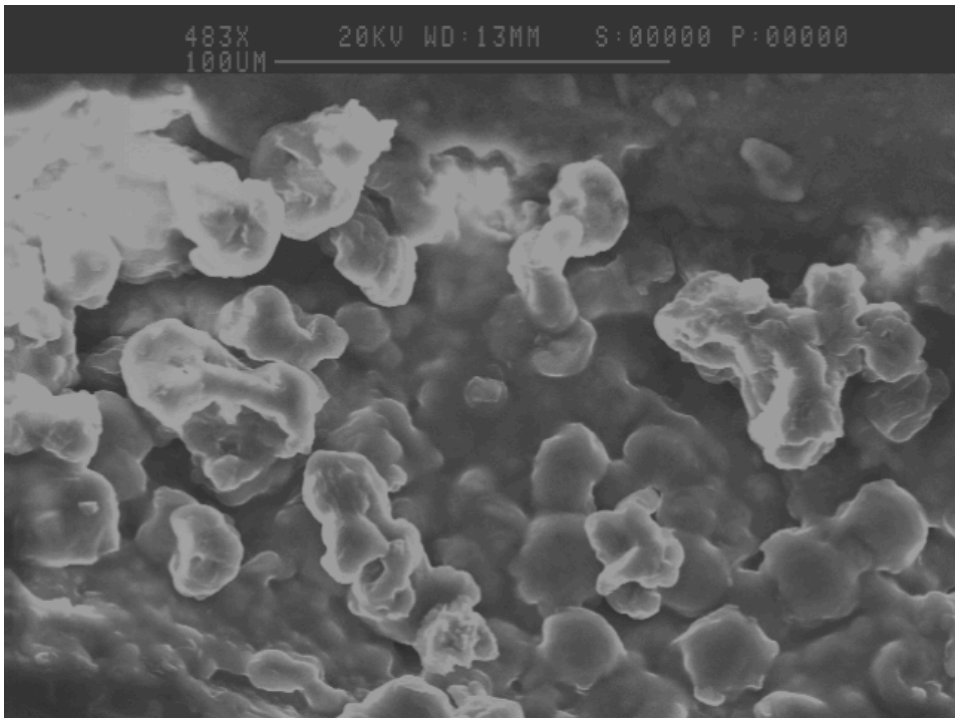

Figure 3.40: Example of bulbous texture from specimen 12 (also exhibited by specimen 17).

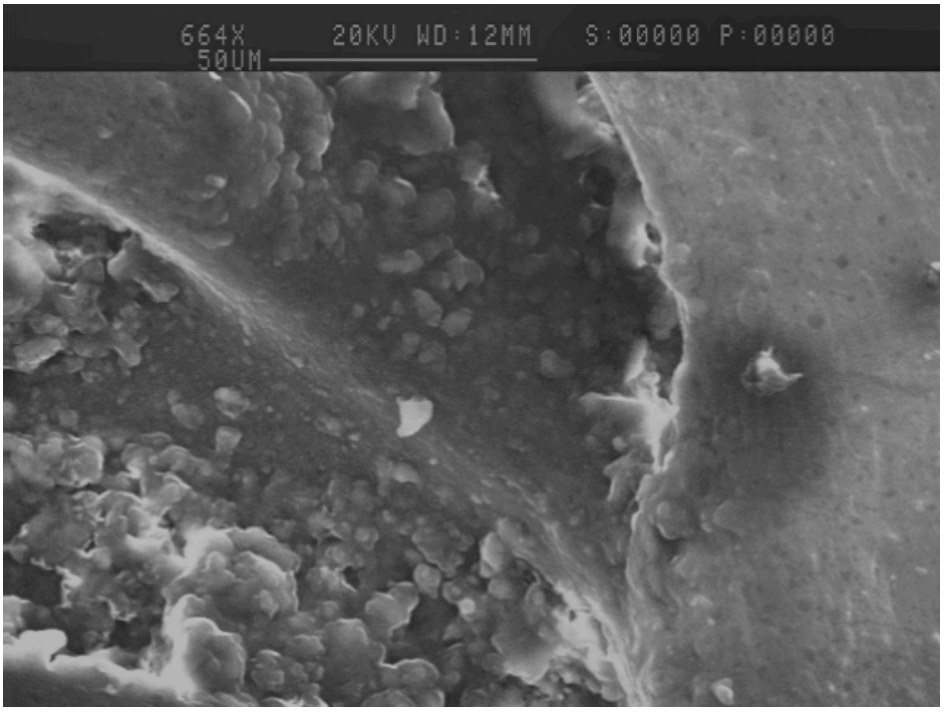

Figure 3.41: Example of bulbous texture in surface opening of specimen 18.

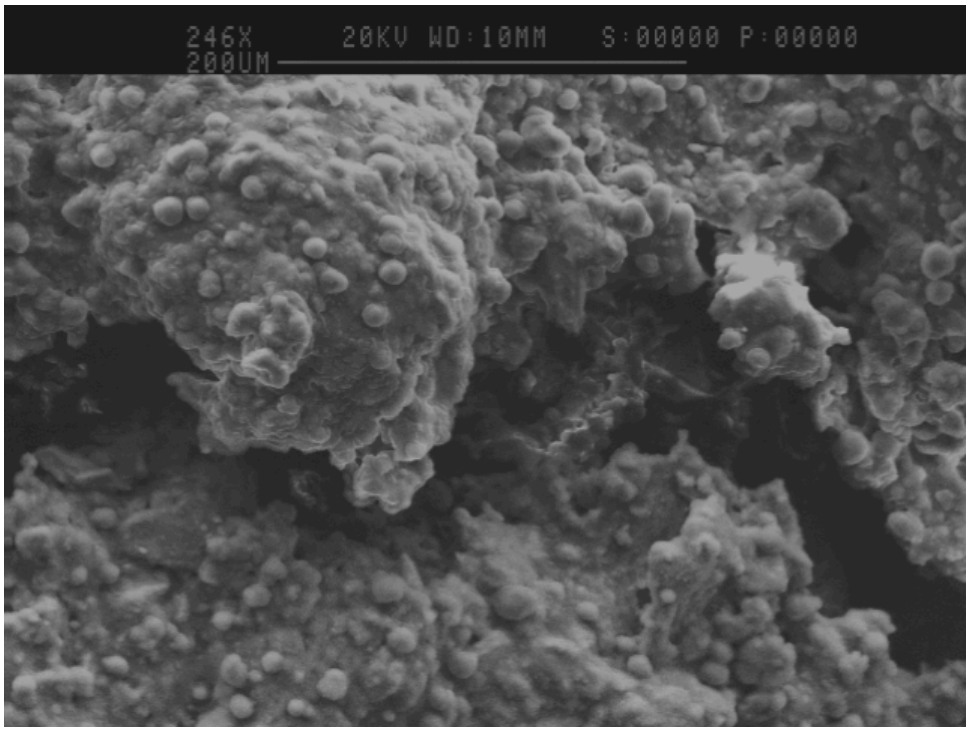

Figure 3.42: Example of bulbous texture from specimen 24 (also exhibited by specimen 23).

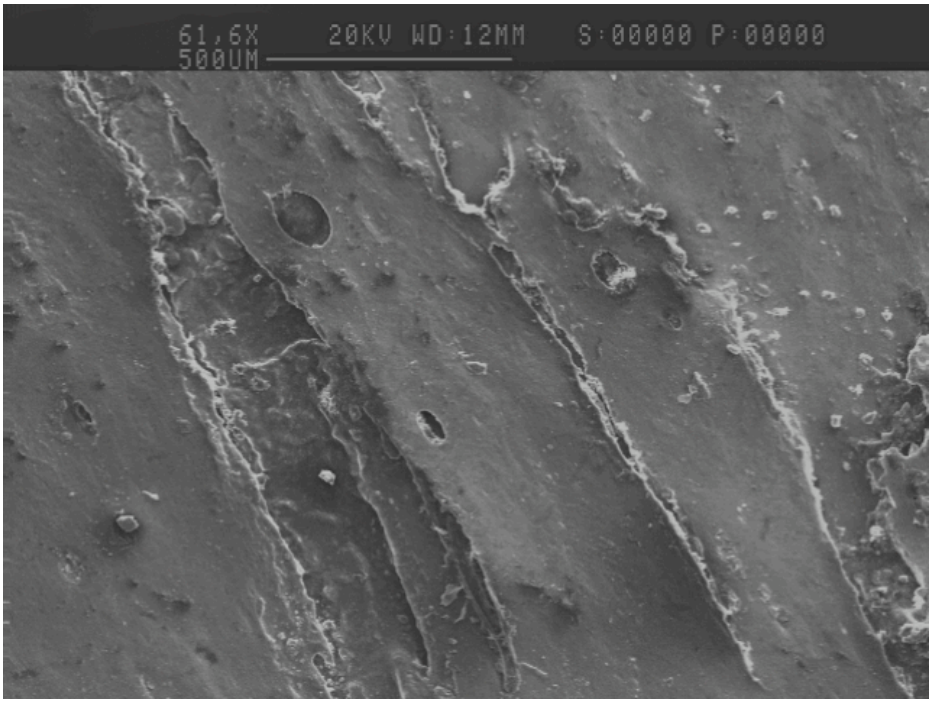

Figure 3.43: Specimen 18 exfoliation of superficial layer.

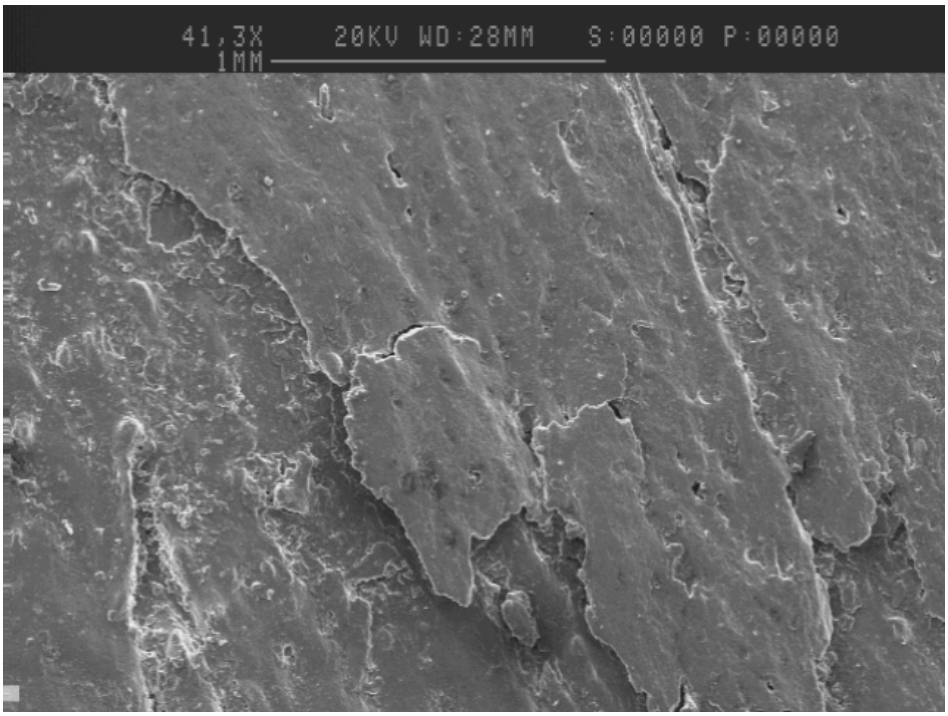

Figure 3.44: Specimen 23 exfoliation.

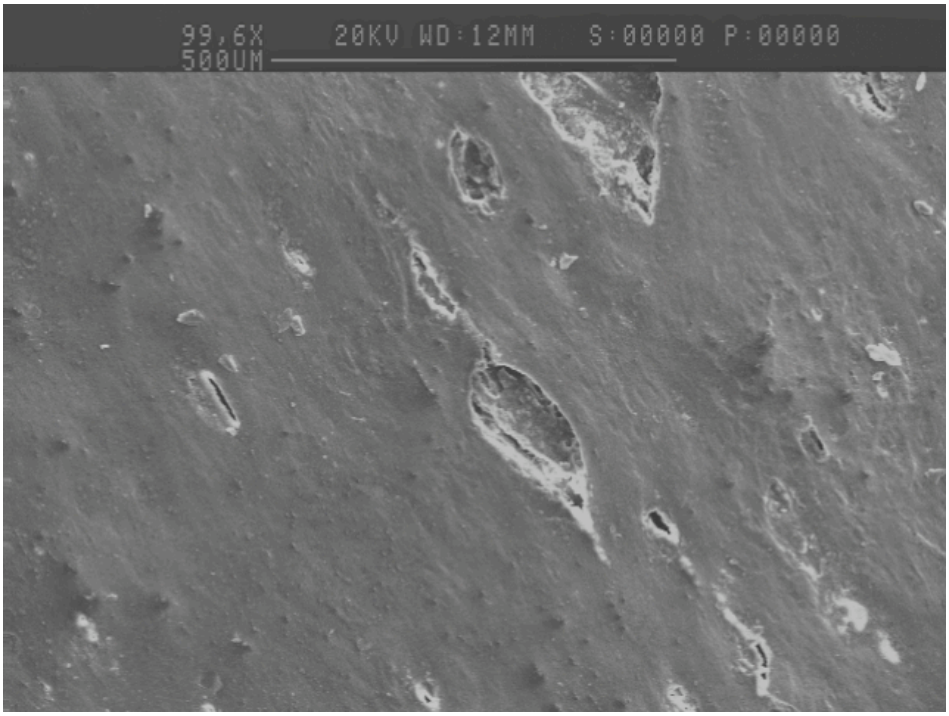

Figure 3.45: Example of hole formation from specimen 18 (also exhibited by specimen 23).

surfaces were typically smooth, or had striations forming into the smooth superficial layer (Figure 3.46), as well as irregular holes (Figure 3.47). Specimen 24's surface had irregular topography due to remaining soft tissue, irregular hole formation, and microfractures (Figures 3.48 to 3.50).

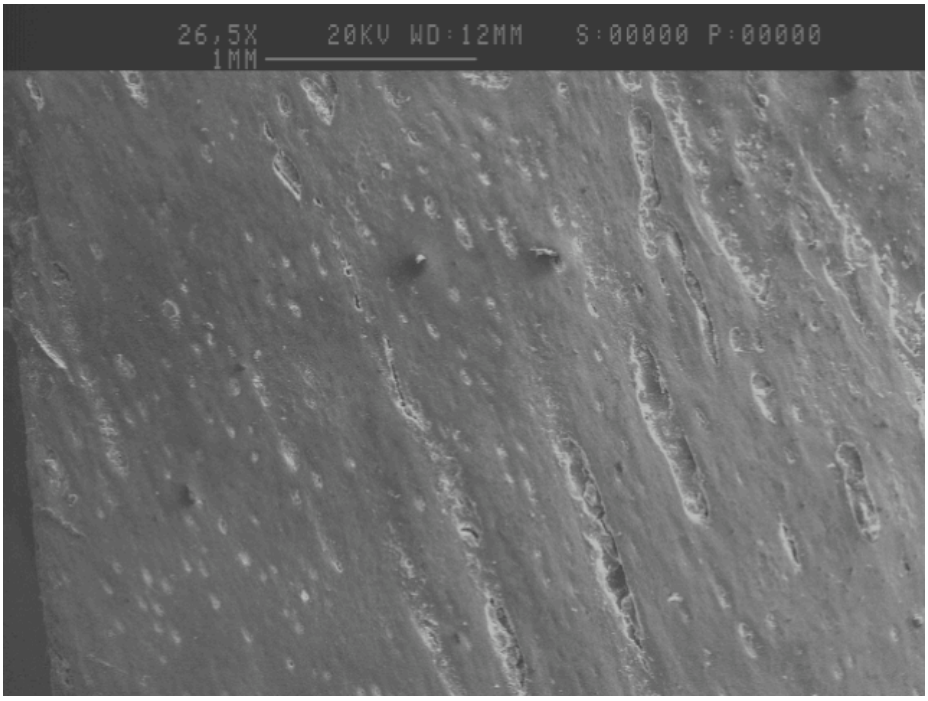

Figure 3.46: Example of striations from specimen 18 (also exhibited by specimen 23).

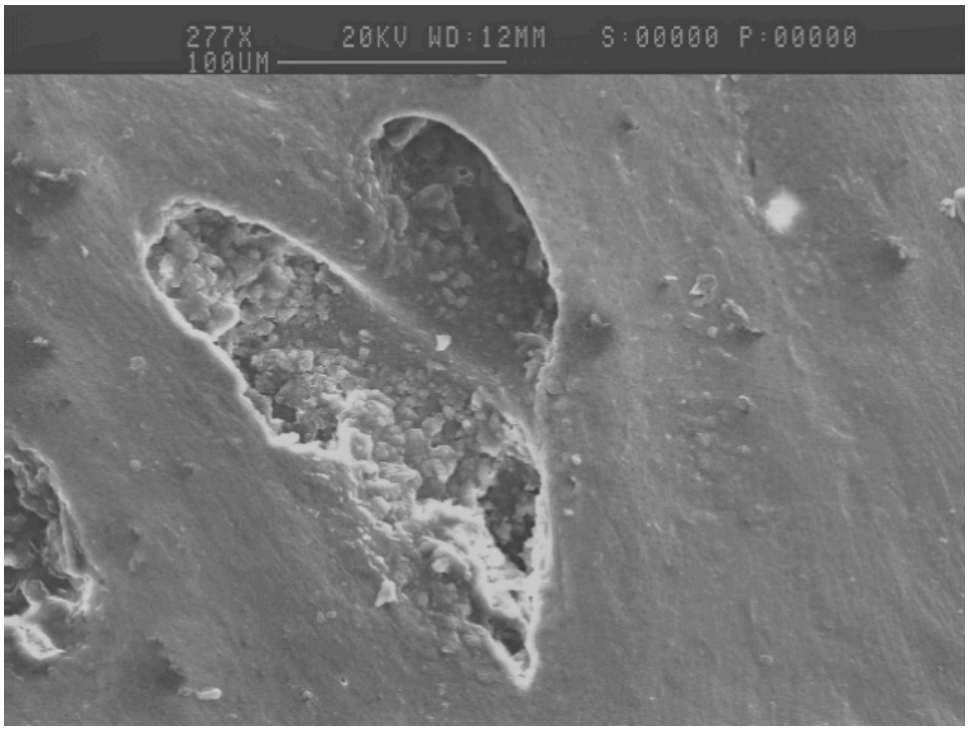

Figure 3.47: Example of irregular hole formation from specimen 18 (also exhibited by specimen 23).

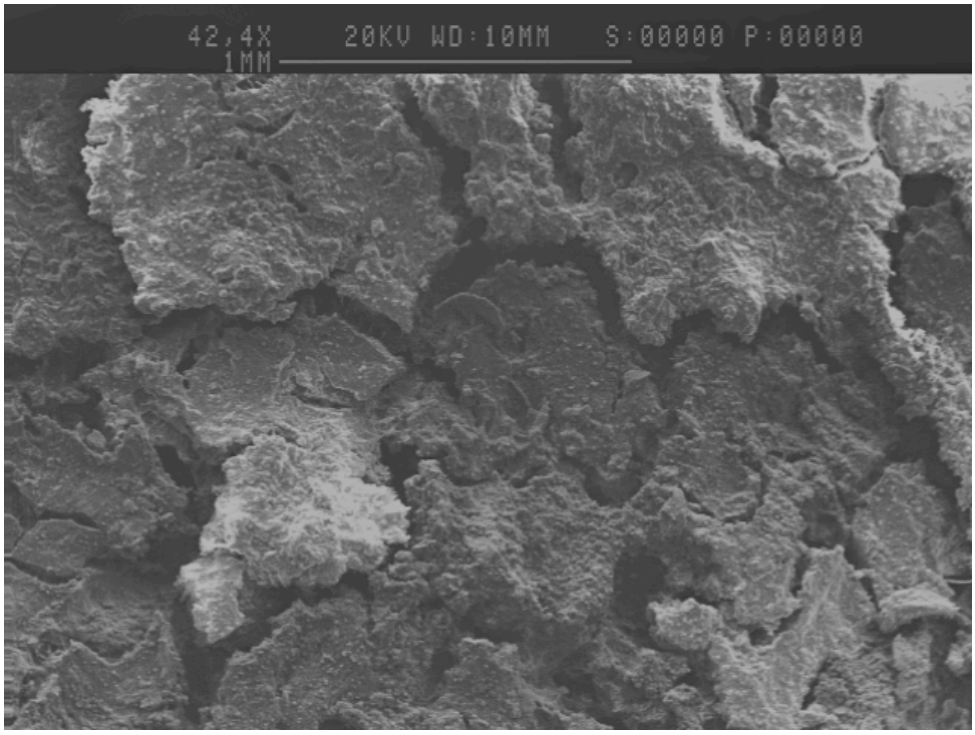

Figure 3.48: Irregular topography of specimen 24.

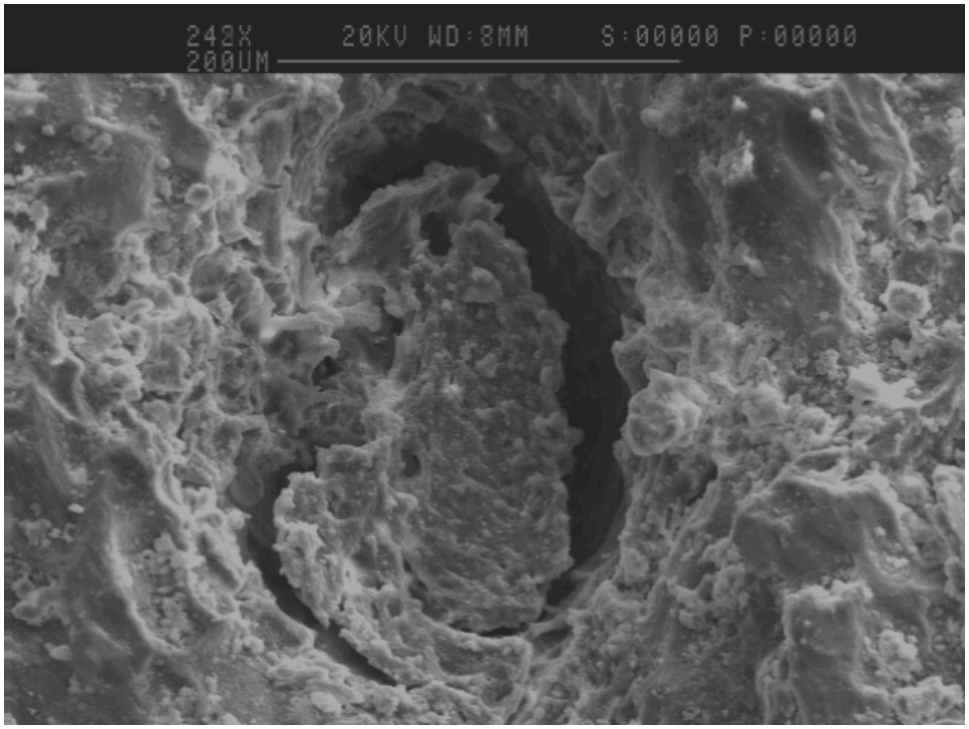

Figure 3.49: Irregular hole formation on specimen 24.

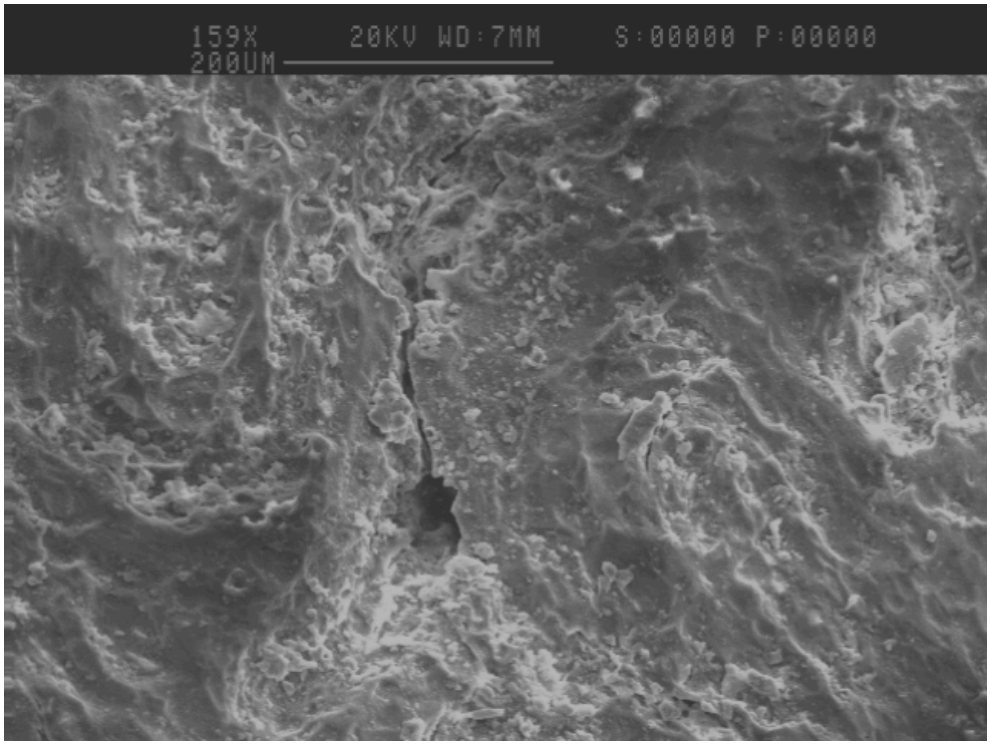

Figure 3.50: Microfracture on specimen 24.

Discussion

4.1 Environmental Data

When interpreting the air moisture environmental data, it is clear that the interpretation of results differs in practicality when comparing the use of vapour density to relative humidity. As demonstrated in Figures 3.01 and 3.02, vapour density had a higher regression coefficient ($r^2 = 0.71167$) with the temperature than did relative humidity ($r^2 = 0.00054$). This is because the calculation of relative humidity, being the amount of water in the air divided by the maximum amount of water the air can hold *at that temperature* (13) generates a value that fluctuates in a variety of ways as the temperature changes. However, vapour density increases with temperature exponentially, since vapour density is the exact concentration of water in the air at any given temperature; this relationship makes sense due to the fact that as the air temperature increases, so does the air's capacity to retain water due to a reduction in the air's density. Vapour density is consequently less misleading to interpret, since a vapour density of a higher value (e.g., $13g/m^3$) indicates there is a higher water concentration in the air than a vapour density of a lower value (e.g., $3g/m^3$), whereas with relative humidity, a higher value does not necessarily indicate that there is a higher amount of water in the air. This is clearly demonstrated in Figures 3.3 and 3.4; on average, the relative humidity outdoors generated higher values than indoors, but when converted to vapour density, it was revealed that there was in fact on average more water in the air indoors than outdoors. From this, we can conclude that vapour density is likely an easier and

more appropriate unit to use when measuring air moisture. This should be noted since currently, vapour density is rarely, if ever used in the literature as a method of moisture measurement in decomposition studies.

4.2 Colour Change Due to Weathering

The photographic CMYK data, when subjected to regression analysis, revealed no significant trends that could be attributed to colour change due to weathering as a function of time. It was predicted that the bones exposed to the most sunlight (sun group 1) would become the palest, and that this would be indicated as a decrease in the black "K" value as well as of the other colours (cyan, magenta, yellow) due to bleaching by the sunlight. Sun bleaching is a phenomenon described in the current literature (12), but is expected to only occur significantly after fairly long periods of weathering. In this study, the weathering periods the specimens were exposed to were relatively short term, since they had only roughly 6 weeks of sun exposure at most. In fact, before maceration, the palest bones of all were the control bones that had absolutely no exposure to any bleaching ultraviolet rays. This is noted in the photographic data and the MSCC results pre-maceration. In addition, after maceration, a seriation was performed that ended up placing the initially "palest" control bones fairly spread apart from each other, indicating no obvious relationship in their colour as previously suspected pre-maceration.

The weak relationship between colour and degree of weathering could be attributed to many factors. As far as the photographic and CMYK data is concerned, day-to-day colour fluctuations are noted and are caused by precipitation (as

demonstrated in Figure 3.16). In addition, the bit of flesh that remained on the bones during the weathering and before maceration promoted the development of mould and bacterial growth, which in itself caused colour fluctuations, and even resulted in multi-coloured bones in some instances, which is also noted in the Munsell results when two or more colours were assigned to the $2cm^2$ area of analysis of a given specimen. Other natural decomposition processes were also at play, such as lipid retention (17), and marrow staining, which changed the bone colour to darker beige or brown colours, or even red/purple colours. When considered simultaneously, all of these transient and uncontrollable factors combined likely lead to the lack of observable trends resulting strictly from the ultraviolet rays of the sun. It is hard to say whether a more significant bleaching trend would be observable in the absence of the uncontrollable factors in this short amount of time, but is worth looking into.

4.3 Macroscopic Analysis

It is stated in the literature that observable taphonomic effects (such as cracking or exfoliation) are not even observable after 150 days of environmental exposure (1), however even before the removal of any persisting soft tissue (with the maceration process), these effects were observable macroscopically in some specimens. Additionally, epiphyseal separation occurred in specimens that were subjected to water submersion, such as the specimens of the moist soil group. The epiphyseal separation was likely due to the specimens being from juvenile pigs whose epiphyseal fusion had not yet completed. Asides from the aforementioned

observations, nothing else was noted pre-maceration. After maceration, more exfoliation and cracking became visible. This may have been due to the effect of dehydration the bones were subject to in the laboratory after the removal of any remaining soft tissue (such as persisting periosteum that may have been preserving the bone). After performing the chi square test on every group, it was determined that the number of cracked specimens after maceration was statistically different than the expected amount, thus indicating that the cracking of specimens is not independent from the group from which they came. This suggests that the environmental effects of the groups had an effect on the cracks that were observed. The results also indicate that the same can be said for the flaking observed before maceration. This provides further indication that in some instances there are significant environmental factors that are playing a role on taphonomic changes in bone in as short of a time period as six weeks.

4.4 Microscopic Analysis

Using the scanning electron microscope allowed for significant distinctions between specimens of varying groups, and revealed the extent to which they underwent degradation. Key signs of bone degradation, such as microfractures, were observed in several specimens, and to a higher degree in those that underwent the most prolonged environmental exposure. For instance, both specimens 4 and 7 that were subject to the longest exposure time (seventeen weeks total), as well as specimen 16 that was in the moist soil group and submerged in water on more than one occasion, had distinct scaly surface textures. The scaly surface is deeper

lamellar bone that has been exposed due to significant weathering. These specimens also had many more surface microfractures in comparison to the other specimens. Due to fluctuating temperatures, this could have occurred from a freeze thaw effect (12). In contrast, the smoother, more easily exfoliated surfaces observed on the control specimens (specimen 23), and in specimen 18 that was on a grass substrate were likely exhibiting such exfoliation since their surface tissue consisted of a more superficial layer of remaining periosteum. It is for this reason that it appears as though the layer of bone flaking off in these specimens is already gone in the first mentioned specimens who underwent higher degrees of weathering. There are also superficial holes forming on the latter specimens, and characteristic cracks (that are discernable from the freeze thaw microfractures observed in the first mentioned specimens) beginning to form as striations on this superficial layer. It should also be noted that the bulbous texture observed on most of the less weathered specimens was due to congealed lipids that accumulated on the bone and in crevices during the maceration process. The irregular topography on control specimen 24 (figure 3.48) is likely persisting soft tissue that failed to be removed during maceration.

Unfortunately, since we were not observing a cross section of the bone, it was difficult to decipher whether some hole formation was due do microboring organisms causing bioerosion of the bone surface. The differences between the boring patterns of these organisms are best observed in cross section (9).

The fact that there was a visible presence of taphonomic change that allowed for the distinction between more or less weathered specimens in as little as six weeks and as much as seventeen weeks on specimens that were fleshed, and thus

also deprived of the degradation effects of endogenous bacteria, contradicts some findings made from other studies that found no observable changes till at least 3 months of exposure with endogenous bacteria on non fleshed bones (11), and other studies that found no taphonomic changes even after 150 days (1).

4.5 Limitations

An important consideration to keep in mind is that non-human *Sus scrofa* humeri were used rather than human bones. Although pig humeri are considered to be a good analogue to human bone (8), the bones in this study were juvenile, and had unfused epiphyses. This lead to observed epiphyseal separation pre-maceration in some specimens, and may have potentially impacted the rate of taphonomic change due to physiological differences between juvenile and mature bone tissue.

The acquirement of the CMYK data from Photoshop© was done as methodically as possible. This was done in an effort to ensure the best representation of the colours of every AOA of each photo was obtained for the regression analysis. However, the average of five points may not have been enough in some cases where there was an extremely high frequency of differing colours in the same AOA. If this method is to be used again, it may be worth it to take the average of more than five points. In addition, the MSCC assessments have a degree of subjectivity to them, and perhaps intraobserver error should also be accounted for by having them done more than once on different occasions, or also repeated by others to compare the similarity of results in order to increase the accuracy of this method.

The maceration of the specimens naturally had a dehydrating effect on the bones due to the removal of their soft tissue, and to a certain degree, this may have contributed somewhat to the resulting increase in exfoliation and cracking observed post-maceration. Additionally, congealed lipids were visible on the specimens on the SEM (figure 3.40 to figure 3.42), as well as a persisting layer of periosteum on the less weathered specimens (figure 3.43 to figure 3.46). In addition, control specimen 24 had remaining soft tissue, as seen on figure 3.48. Since there was apparently remaining periosteum, lipids, and soft tissue on the less weathered specimens, perhaps they should have been subjected to a higher degree of maceration by either increasing their time in the heated solution, or by increasing the concentration of the Tergazyme agent. The fact that a higher degree of maceration is required for the less weathered specimens is indicative of the lesser degree of weathering they experienced, and this is notable from their surface microstructure.

Conclusion

5.1 Conclusions

This study's weather results indicate the potential superior practicality of using vapour density as a means of measuring air moisture rather than using relative humidity. The former method should be adapted in future studies to facilitate interpretation of weather data and reduce potential errors.

Additionally, bone colour change during short-term sub aerial weathering is not only observable, but also highly transient due to several factors, such as bacterial and mould growth, precipitation, bone staining from marrow and lipid retention, and possibly even other factors not previously considered. Due to these environmental factors, our colour results showed no discernable trends, and also made it difficult to track colour change due solely to ultraviolet sun rays.

Key taphonomic changes, being flaking and cracking, were already observable within 6 weeks to a few months of environmental exposure in certain specimens, thus contradicting the results of previous studies (1, 11). When viewed with a scanning electron microscope, further distinctions can be made between specimens of varying degrees of environmental exposure. These resulted in taphonomic differences due to weathering, such as differences in surface texture, in the amount of cracks observed, in the exfoliation of the most superficial layer of tissue, and even in hole formation (due to the differential persistence of the superficial tissue layers, such as the periosteum).

5.2 Forensic Relevance

The results obtained in this study may be of forensic interest when analyzing skeletonized remains. It is important to keep the above-mentioned findings in mind, since taphonomic effects were already observable on fleshed specimens in as little as six to seventeen weeks, thus indicating that bone undergoes quicker taphonomic degradation than previously thought (1, 11). There was discernable variability in surface texture, exfoliation, and amount of microfractures present depending on which microenvironment the specimen was exposed to. The use of an SEM allows these features to be quite readily identified. With the consideration of these findings, methods incorporating an SEM to identify surface microstructural changes for remains having gone through short-term subaerial exposure may be developed for post mortem interval (PMI) estimations.

5.3 Future Research

Further research may be warranted in order to examine the impact of the above studied environmental effects individually on bone taphonomic changes, such as sunlight exposure, vapour densities, temperatures, and substrates. In order to isolate the direct effect of these environmental factors, the factors should be studied separately and with as much control as possible on surrounding interfering factors. For instance, to analyze the effects of ultraviolet rays on bone colour specifically, bones could be placed under UV lamps with varying degrees of ultraviolet light in a laboratory where as many potentially interfering aspects of the environment as possible are controlled. The results could then be compared to specimens that were

left out in more typical uncontrolled environmental conditions so that accurate identification of the cause of the occurring features can be performed, and information regarding what type of environment the remains were exposed to can be gained. This could be done sub aerially on both macerated, as well as fleshed specimens (that may have soft tissue remaining, as was the case in this study) to see how this will impact the bone's susceptibility to the aforementioned environmental taphonomic factors. Finally, continuous SEM analysis could be performed throughout the study rather than only at the end in order to track these changes as they happen. That way, the accuracy of potential PMI estimations can be increased.

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